# A HANDBOOK OF
# SUGAR ANALYSIS

A PRACTICAL AND DESCRIPTIVE TREATISE
FOR USE IN
RESEARCH, TECHNICAL AND CONTROL LABORATORIES

BY

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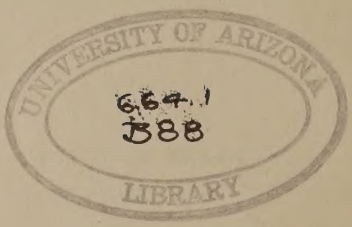


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DEDICATED

TO HIS TEACHER,

GEH.-RATH PROF. DR. B. TOLLENS,

OF GÖTTINGEN UNIVERSITY,

AS A TOKEN OF GRATITUDE AND ESTEEM,

BY THE AUTHOR

18736

# PREFACE

THE subject of sugar analysis, which a generation ago was limited to determinations of density, specific rotation and reducing power, has greatly expanded within the past twenty-five years. Instruments of greater accuracy have been devised, old methods have been improved and new methods have been discovered. In the present volume the purpose of the author has been to give a rather wide, but a by no means complete, selection of the more recent methods of sugar analysis and at the same time to retain the more important features of the older textbooks.

The range of sugar analysis is so broad that in the selection of methods the author has been guided largely by his own experience in various research, technical and control laboratories. While the particular methods chosen for description may not in all cases meet with general approval it is hoped that the underlying principles of sugar analysis have been covered sufficiently to enable the chemist to make his own applications and modifications. References to special works and original articles will assist the chemist in case he desires to follow some special line of investigation more fully.

Next to the knowledge of a method the most important fact which the student of sugar analysis must acquire is the knowledge of this method's limitations. The great susceptibility of the sugars to chemical changes and to variations in specific rotation, reducing power and other "constants" is a factor which the sugar chemist must always bear in mind. The prescribed methods of analysis are usually too silent upon these points, and the inexperienced chemist often proceeds to make general use of a formula or method which has only a limited applicability. The author has endeavored to correct this tendency by including with the description of each method a brief account of its applicability and limitations.

In the examination of sugar-containing materials the problems of analysis are much simplified by a knowledge of what one may expect to find. The author has felt that a work upon sugar analysis is not complete without some description of the sugars themselves. In Part II of the present volume, he has therefore included a brief

account of the occurrence, methods of preparation, properties and reactions of the different sugars and their allied derivatives. Brief references are also made to methods of sugar synthesis; the latter play such an important part in the separation and isolation of the rarer sugars that the sugar analyst is not fully equipped without some knowledge of synthetic processes.

The principal textbooks and journals which have been consulted in preparing the present volume are named in the Bibliography. The author's obligations to these are indicated in most cases by the footnotes. In reviewing original papers, the abstracts and references contained in Lippmann's "Chemie der Zuckerarten" and his "Berichte über die wichtigsten Arbeiten aus dem Gebiete der reinen Zuckerchemie," published semiannually in "Die Deutsche Zuckerindustrie," have been of invaluable service.

In concluding his task, which has extended with many interruptions over a period of five years, the author desires to thank the many friends and coworkers who, by their help and encouragement, have greatly lightened his labors.

Special obligations are due to Dr. C. S. Hudson for reviewing the section upon mutarotation and to Prof. H. C. Sherman for suggestions upon methods for determining diastatic power. Acknowledgement is also made of courtesies extended by Mr. A. H. Bryan and by Mr. G. W. Rolfe.

For the use of cuts contained in Dr. G. L. Spencer's "Handbook for Cane Sugar Manufacturers" and in A. E. Leach's "Food Inspection and Analysis" the author owes an acknowledgment to the authors of these books and to his publishers Messrs. John Wiley & Sons. To the latter also he would express his appreciation of the hearty support which has been given and of the generous consideration which has been shown for the many delays incident to the completion of the work.

New York, N. Y., August, 1912.

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## PERIODICALS


| *Abbreviation* | *Title* |
|---|---|
| Am. Chem. Jour | American Chemical Journal. |
| Am. Sugar Ind | American Sugar Industry. |
| Analyst | Analyst. |
| Ann | Annalen der Chemie (Liebig's). |
| Ann. chim. phys | Annales de chimie et de physique. |
| Archief Java Suiker Ind | Archief voor de Java Suiker Industrie. |
| Archiv Pharm | Archiv der Pharmazie. |
| Ber | Berichte der deutschen chemischen Gesellschaft. |
| Biochem. Zeitschrift | Biochemische Zeitschrift. |
| Bull. assoc. chim. sucr. dist | Bulletin de l'association des chimistes de sucrerie et de distillerie de France et des colonies. |

| *Abbreviation* | *Title* |
|---|---|
| Bull. soc. chim. | Bulletin de la société chimique de France. |
| Centralblatt | Chemisches Centralblatt. |
| Centrbl. Zuckerind. | Centralblatt für die Zuckerindustrie. |
| Chem. News | Chemical News and Journal of Physical Science. |
| Chemiker-Ztg. | Chemiker-Zeitung. |
| Compt. rend. | Comptes rendus hebdomadaires des seances de l'academie des sciences. |
| Deut. Zuckerind. | Die Deutsche Zuckerindustrie |
| Dingler's Polytech. Jour. | Dingler's Polytechniches Journal. |
| Int. Sugar Jour. | The International Sugar Journal. |
| J. Am. Chem. Soc. | Journal of the American Chemical Society. |
| J. Chem. Soc. | Journal of the Chemical Society (London). |
| J. fabr. sucre | Journal des fabricants de sucre. |
| Jour. f. Landwirtsch. | Journal für Landwirtschaft. |
| J. Ind. Eng. Chem. | Journal of Industrial and Engineering Chemistry. |
| J. pharm. | Journal de pharmacie. |
| J. pharm. chim. | Journal de pharmacie et de chimie. |
| J. prakt. Chem. | Journal für praktische Chemie. |
| J. Soc. Chem. Ind. | Journal of the Society of Chemical Industry. |
| La. Planter | The Louisiana Planter and Sugar Manufacturer. |
| Land. Vers.-Stat. | Die landwirthschaftlichen Versuchs-Stationen. |
| Monatshefte | Monatshefte für Chemie. |
| Mon. scient. | Moniteur scientifique. |
| Neue Zeitschrift | Neue Zeitschrift für Rübenzuckerindustrie. |
| Oest.-Ung. Z. Zuckerind. | Oesterreichisch-Ungarische Zeitschrift für Zuckerindustrie und Landwirthschaft. |
| Pflüger's Archiv | Pflüger's Archiv für die gesammte Physiologie der Menschen und der Thiere. |
| Pogg. Ann. | Poggendorff's Annalen. |
| Proceedings A. O. A. C. | Proceedings of the Association of Official Agricultural Chemists. |
| Proceedings Int. Cong. App. Chem. | Proceedings of the International Congress of Applied Chemistry. |
| Rec. trav. Pays-Bas | Recueil des travaux chimiques des Pays-Bas. |
| Sitzungsber. Wiener Akad. | Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, Wien. |
| Stammer's Jahresbericht | Stammer's Jahresbericht über die Untersuchungen und Fortschritte auf dem Gesamtgebiete der Zuckerfabrikation. |
| Sucrerie Belge | La Sucrerie Belge. |
| West Indian Bull. | West Indian Bulletin. |
| Wochenschr. f. Brauerei | Wochenschrift für Brauerei. |
| Z. analyt. Chem. | Zeitschrift für analytische Chemie. |
| Z. angew. Chem. | Zeitschrift für angewandte Chemie. |
| Z. Instrument. | Zeitschrift für Instrumentenkunde. |
| Z. physik. Chem. | Zeitschrift für physikalische Chemie. |
| Z. physiol. Chem. | Zeitschrift für physiologische Chemie. |
| Z. Spiritusind. | Zeitschrift für Spiritusindustrie. |
| Z. Unters. Nahr. Genussm. | Zeitschrift für Untersuchung der Nahrungs- und Genussmittel. |
| Z. Ver. Deut. Zuckerind. | Zeitschrift des Vereins der Deutschen Zuckerindustrie. |
| Z. Zuckerind. Böhmen | Zeitschrift für Zuckerindustrie in Böhmen. |

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# PART I

## PHYSICAL AND CHEMICAL METHODS OF SUGAR ANALYSIS

# SUGAR ANALYSIS

## CHAPTER I

### SAMPLING OF SUGAR AND SUGAR PRODUCTS

In the analysis of sugars and sugar products, special stress must be laid upon the correctness of sample. Accuracy in analytical details is of no value unless the portion of substance weighed out for examination is an accurate sample of the entire lot of product in question. While the chemist is not always charged with the supervision of sampling, he should, nevertheless, acquaint himself so far as possible with the history of his product before it is received. In this way he may often explain differences which might otherwise be attributed to mistakes of analysis. A few introductory pages devoted to the general subject of sampling may, therefore, not be amiss.

The best illustration of methods of sampling, and of the errors connected therewith, is furnished by raw cane sugar. The sampling of this commodity is selected first and discussed in somewhat fuller detail.

#### Sampling of Raw Sugars

The raw sugar imported from the various sugar-producing countries comes in a great variety of forms. Centrifugal sugar, from Cuba, Porto Rico, and most of the West Indian Islands, comes in 300-lb. jute bags; sugar from the Hawaiian Islands comes in 125-lb. bags; sugar from Java comes either in bags or large cylindrical baskets weighing from 500 to 700 lbs.; sugar from the Philippines comes in small wicker mats weighing about 50 lbs.; Muscovado sugars, which are purged by draining and contain much molasses, come usually in large hogsheads. In addition to the above forms of package, sugars come occasionally in boxes, barrels, grass mats, ceroons, and other receptacles.

The need for carefully prescribed rules in sampling sugar becomes at once self-evident when we consider the different forms of the package and the exceedingly variable character of the sugar which may be con-

tained therein. The sugar, for example, may contain lumps of higher or lower polarization than the finer part of the product; the sugar may also retain considerable amounts of molasses, sometimes as high as 30 per cent, which drain during transit or storage and form the " foots " at the bottom of the package. The difference in composition between the top and bottom layers of a hogshead of Muscovado sugar, which is a kind that "foots" easily, is very marked. In addition to the differences in composition of sugar within the single packages are the differences in composition between different packages of the same lot. These differences may be the result of manufacture; they may also result when no dunnage is used for covering the bottom of the

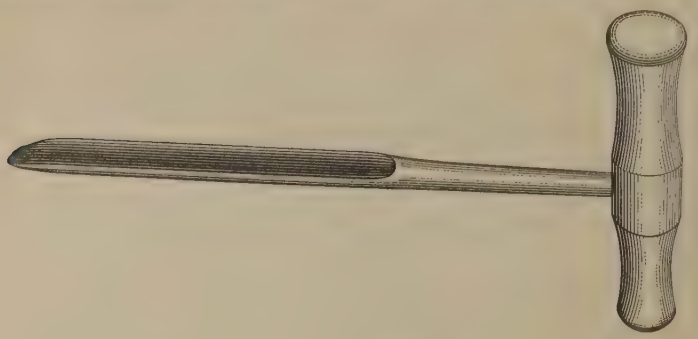


Fig. 1. — Trier for sampling sugar.

holds of the ships used for transport, with the result that the bottom tiers of sugar may be damaged through absorption of bilge water. In many cases the top tiers of sugar suffer the damage, as when sugars sweat beneath the hatches; the vapors from the warm sugar rise, condense, and then drop back upon the upper layers of the cargo. If the packages of sugar run unevenly it is difficult to secure a representative fraction unless every container is sampled. The most approved method of sampling at present is to take a specimen of sugar so far as possible from every package.*

Sugar is sampled in the same way as fertilizers and many other commodities, — by means of a trier. This implement (Fig. 1) consists of a long pointed rod of steel with a groove or spoon upon one side. A

* For a discussion of this and other points pertaining to methods of sampling raw sugar in different countries see paper by F. G. Wiechmann (Int. Sugar Journ., 9, 18–28) read before the Fifth Meeting of the International Commission for Uniform Methods of Sugar Analysis, Bern, 1906.

thrust of the trier into the package forces the sugar along its pathway tightly into the bowl of the spoon; the sugar thus adhering, after the trier is withdrawn, is removed by the thumb, or by means of a scraper, into a covered bucket, and the process is continued until a sufficient number of packages have been sampled to constitute a mix; this number may vary, according to the size of lot and kind of sugar, from one package to several thousand. The practice of the New York Sugar Trade is to mix twice daily, and in no case is a sample to remain unmixed over night.

It is of course important that the triers of the different workmen who are sampling a given lot of sugar should be exactly alike, especially as regards the dimensions of the spoons. The specifications of the United States Treasury Department Regulations* are very explicit upon this point and give the following dimensions of the short, long, and barrel triers.

TABLE I

*Giving Dimensions of Triers for Sampling Sugar*

| | Short trier. | Long trier. | Barrel trier. |
|---|---|---|---|
| | Centimeters. | Centimeters. | Centimeters. |
| Length over all | 40.6 | 152.4 | 104.0 |
| Length of spoon | 22.9 | 132.1 | 91.4 |
| Length of shank | 17.8 | 20.3 | 12.7 |
| Length of handle | 26.7 | 38.1 | 30.5 |
| Width of spoon | 2.7 | 2.5 | 2.5 |
| Depth of spoon | 0.8 | 1.3 | 1.1 |
| Diameter of handle | 3.8 | 3.8 | 3.8 |

According to the United States Treasury Department Regulations,† "sugar in hogsheads and other wooden packages shall be sampled by putting the long trier diagonally through the package from chime to chime, one trierful to constitute a sample, except in small lots, when an equal number of trierfuls shall be taken from each package to furnish the required amount of sugar necessary to make a sufficient sample. In the sampling of baskets, bags, ceroons, and mats the short trier shall be used, care being exercised to have each sample represent the contents of the package."

It is necessary in sampling to keep the triers always clean; the sticking of sugar to the bowl of the spoon is especially annoying with some

* *Regulations* governing the weighing, taring, sampling, classification, and polarization of imported sugars and molasses. U. S. Treasury Department, Division of Customs, Document No. 2470, Art. 5.

† Loc. cit., Art. 6.

kinds of sugar under certain atmospheric conditions of humidity. The surface of the metal should be smooth and bright; the United States Treasury Regulations attach a penalty in case of samplers who neglect this precaution. When ready for making the composite sample, the contents of the sugar bucket are thoroughly mixed; the cans and bottles to receive the sample are compactly filled, labeled, and sealed, after which they are sent to the chemists who are to make the polarizations.

The general rule in sampling sugar is that the package shall be stabbed at the middle to the center, and if this practice is conscientiously followed it will give no doubt as fair a sample as can be secured under the hurried conditions of discharging a cargo. There are times, however, when it is impossible to follow this rule. Sugar which has remained for a long time in storage will sometimes solidify upon the approach of cold weather to a hard mass of material resembling concrete, a circumstance due to the evaporation of moisture and cementing together of the grain. A trier is almost useless under these conditions and such sugar is rarely sampled properly. The sugar broken, or chipped off, by the trier from the outside of the package is not a correct sample. A pickaxe is sometimes resorted to with hard sugar in order to open a passage for the trier; this is much better than just skimming the outside, but is far from satisfactory.

To eliminate so far as possible the errors of personal equation in sampling, the practice of the New York Sugar Trade is for the samplers of buyer and seller to work alternately hour by hour; the one party in the interval of rest exercising a control upon the operations of the other. The tendencies to draw too high and too low from the package are thus counterbalanced and the personal errors equalized. This method seems as good as any that can be devised.

The liability of change in composition of the product during sampling is an exceedingly important factor in the valuation of any commodity, and more important perhaps in the case of sugar than almost any other staple. Raw cane sugar upon exposure to the air may either absorb or lose moisture according to the conditions of atmospheric humidity. If the latter be very high or low, and the sugar be exposed to the air for any great length of time during drawing or mixing the sample, a considerable error may be introduced into the composition of the product. The buckets, which hold the samples for mixing, should always be kept tightly covered; this precaution will reduce the errors from absorption and evaporation to a large extent, although with present methods of sampling the errors from this source will never be

completely eliminated. On rainy days sugar is rarely sampled at the pier, and this is a wise precaution, considering the rapidity with which sugar absorbs moisture from a saturated atmosphere. No matter how pure the sugar, there will be absorption under such conditions, the amount of moisture taken up depending upon the initial dryness of the sugar, the fineness of the grain and the hygroscopic character of the impurities present.

If a layer of sugar be placed in a dish over water under a closed bell jar, it will soon absorb moisture enough to liquefy, and, according to the phase rule, this absorption of moisture will continue until the pressures of water vapor for solution and atmosphere are the same. Theoretically this limit is infinity, and if the dish under the bell jar be weighed from day to day it will be found that the liquefied sugar will continue to attract moisture as long as one cares to follow the experiment.

If the atmosphere is not completely saturated, the absorption of moisture by the sugar is less rapid, and with further decrease in humidity a point of equilibrium is soon reached where there is neither absorption nor evaporation. This point of equilibrium, which represents equality of vapor pressure between the moisture of the sugar and the air, is different for different sugars. With still further decrease in humidity the sugar begins to give up moisture, the rate of loss increasing as the percentage of saturation in the air becomes less and less.

In the following table the percentages of moisture which different sugars gain or lose at 100 per cent relative humidity and at 60 per cent relative humidity are given, and the changes in moisture content at the point of equilibrium. Two grams of sugar were spread in a thin layer upon a watch glass and the change in weight noted after regular intervals of time in one case over water under a bell jar, and in the other case upon exposure to the open air. The temperature of experiments was 20° C.

TABLE II

*Showing Variations in Moisture Content of Sugars*

| Kind of sugar. | Grain. | Polarization. | Moisture in sugar. | Gain first hour, 100 per cent humidity. | Change first hour, 60 per cent humidity. | Total change at point of equilibrium. | Humidity at equilibrium. | Residual moisture at equilibrium. |
|---|---|---|---|---|---|---|---|---|
| | | | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. |
| Granulated..... | Fine | 99.85 | 0.10 | 1.78 | +0.03 | +0.01 (2 hours) | 56 | 0.11 |
| Peruvian..... | Large | 98.40 | 0.35 | 1.09 | −0.09 | −0.14 (4 hours) | 56 | 0.21 |
| Porto Rico..... | Medium | 96.40 | 1.31 | 1.40 | −0.54 | −0.73 (2 hours) | 62 | 0.58 |
| Philippine mats. | Fine | 87.45 | 3.12 | 1.80 | −0.68 | −1.25 (6 hours) | 56 | 1.87 |
| Cuban molasses | Large | 82.75 | 4.85 | 1.12 | −1.00 | −2.42 (24 hours) | 59 | 2.43 |

After the point of equilibrium was reached upon exposure of the above sugars to the air, no change in weight was noted as long as the temperature and relative humidity remained unchanged; with fluctuations in the latter corresponding gains and losses were always observed in the weight of the sugars.

As to the absorption of moisture by sugars under excessive humidity, no relationship can be traced in the above table between composition and rate of absorption. The refined granulated sugar and the low-grade mats have equally high absorptive powers and the high-grade Peruvian crystals and the Cuban molasses sugar equally low absorptive powers. If the grain of these sugars is compared, however, it will be seen that the Peruvian crystals and molasses sugar of low absorptive power have the largest grain and that the granulated sugar and mat sugar of highest absorptive power have the smallest grain, so that the physical condition of the sugar is a very important factor in the influences which bear upon absorption.

As to the evaporation of moisture from sugars under diminished humidity, the table shows a very definite relationship between composition and rate of evaporation, this rate being, as would be supposed, roughly proportional to the initial moisture content of the sugar. The percentage of residual moisture in a sugar at the point of equilibrium is a function of the hygroscopic power of the non-sugars, and is greatest with the sugars of lowest purity (highest molasses content).

The point of greatest importance, in the bearing which these results have upon the changes in composition of sugar during sampling, is that the gain or loss in weight through absorption or evaporation of moisture is most rapid at the beginning. A comparison recently made by the author of the changes in moisture content which sugars undergo upon exposure to the air shows that the relationship between time and loss or gain in moisture follows approximately the well-known equation for slow reactions, $k = \frac{1}{t} \log \frac{a}{a - x}$, in which $a$ is the total change in moisture content at the point of equilibrium, $x$ the loss or gain in weight at the end of any given time $t$, and $k$ the coefficient of velocity, which is a constant quantity for each kind of sugar under fixed conditions of temperature and humidity.

The assumption is frequently made by samplers of sugar that the errors from absorption and evaporation of moisture by the sample will equalize one another in the long run. This, however, is far from being the case. The percentage of moisture in the ordinary grades of raw cane sugar is considerably above the equilibrium point for the average

relative humidity at the port of New York. It should be stated, however, that the loss from evaporation under the prescribed conditions of sampling is nowhere near as great as that in the above experiments, where the sugars were exposed to the open air in a thin layer. The error, however, does exist, and unless due care is exercised by the sampler there will be a very noticeable difference in the test.

Another occasional source of error in the sampling of sugar is the introduction into the sample of particles of bag, basket, mat, shavings of barrels, etc., which are introduced from the package by the trier. The error from this cause is usually trifling; there are times, however, when it may be considerable. Such fragments of extraneous matter do not belong to the sugar, and it devolves upon the chemist to eliminate these as far as possible before weighing out the sugar for polarization. In removing foreign material from sample sugar the chemist must carefully discriminate, however, between trash which belongs to the sugar and refuse which is introduced during sampling.

In addition to removing trash, the chemist must complete the mixing of the sample. Lumps must be crushed and thoroughly incorporated with the rest of the sample. Even samples of sugar, which are well mixed at the point of sampling, must be mixed again at the laboratory owing to the segregation of foots at the bottom of the can or bottle. A neglect of such mixing of the sample in the laboratory is a cause of frequent differences between the results of different chemists. This mixing of the sample must be done with the utmost dispatch in order to avoid the errors due to absorption or evaporation already mentioned. Mixing of the sample upon paper or other porous substance which would absorb moisture is especially to be avoided. The method of mixing followed by the New York Sugar Trade Laboratory is as follows:

When samples are brought into the laboratory during freezing weather, the cans or bottles are first allowed to come to approximately the room temperature before opening and mixing. This is done to guard against condensation of moisture upon the cold sugar, which would lower the polarization. The sugar is poured out from the can upon a clean sheet of plate glass, all pieces of bagging, baskets, mats, etc., are removed, and the sample is thoroughly mixed with a clean steel spatula. Lumps are reduced by means of a porcelain roller and incorporated with the rest of the sample. The plate glass and porcelain roller are cleaned and wiped perfectly dry each time before using. The reduction of lumps is of greatest importance in securing uniformity of sample; the difference in polarization between the lumps and the fine portion of some sugars has been found to vary several per cent.

The can from which the sugar was taken is then filled about three-fourths full, the excess of sugar upon the plate being discarded. By leaving a little empty space in the can, the weighing out of the sample by the chemist is facilitated.

### Sampling of Juices, Sirups, Molasses, and Liquid Sugar Products

The sampling of juices, sirups, molasses, and other liquid sugar products involves no special difficulties provided the material be of even composition throughout the body of the container. A large glass or metal tube may serve for withdrawing samples of molasses, etc., from the bungholes of hogsheads, barrels, and casks, when other means are not available. Containers of different capacity should be sampled separately, and in making composite samples each individual fraction should be proportionate to the total amount of material from which it was drawn.

The regulations of the United States Treasury Department* governing the sampling of molasses are as follows: "In drawing samples of molasses, care shall be taken to secure a fair representation and an equal amount of the contents from each package. Packages of the same size shall be sampled in groups of not more than 25; samples from all of the packages of each group being put into a bucket. An accurate tally shall be kept and with each bucket shall be reported the number of packages the samples therein represent. The dock list accompanying the sample buckets shall convey the same information and account for every package of the mark. Packages of different size, although invoiced and permitted under the same mark, shall be separately sampled, tested, and returned for classification. Molasses discharged from tank vessels shall be sampled as it is pumped from the tanks, a sample of uniform quantity being drawn at either regular intervals of approximately fifteen minutes or for every 5000 gallons discharged."

In sampling the juices from mills and diffusion batteries in sugar factories, various automatic sampling devices have been devised for the purpose of securing a sample of the main body of juice at each instant of time. Coomb's drip sampler (Fig. 2) is an illustration of such a device. A defect of such automatic contrivances is that they do not always give a flow of sample proportionate to the total amount of juice.†

* Loc. cit., Art. 16.

† A very efficient automatic liquid sampler is described by G. L. Spencer in the J. Ind. Eng. Chem., **2**, 253; **3**, 344.

In grinding sugar cane, when it is desired to test the work of maceration or to determine the relative efficiency of each mill, the juices from the several sets of rollers are sampled and analyzed separately, the results of the work enabling the chemist to calculate the composition of the so-called "normal" juice or to determine the extracting power

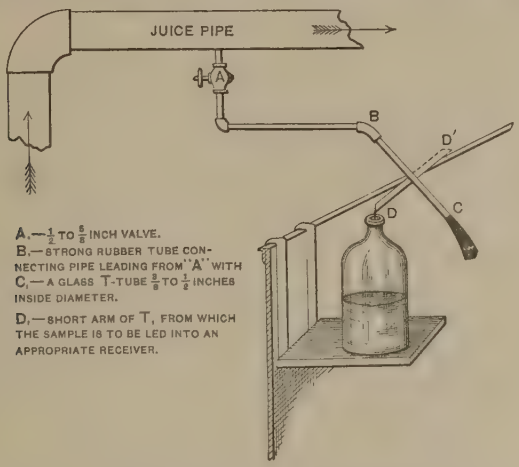



Fig. 2. — Coomb's apparatus for sampling sugar juices.

of each mill. This phase of sampling belongs, however, to the subject of sugar-house control, and the chemist is referred to the special treatises by Spencer, Prinsen Geerligs, Deerr, and others.

### Errors of Sampling Due to Segregation of Sugar Crystals

A serious error in the sampling of liquid sugar products is often occasioned by the crystallization and separation of sugar within the container. The deposition of sucrose crystals from molasses, and from maple, cane, and sorghum sirups, is an example of this; the granulation of strained honey through separation of crystallized glucose is another illustration. Containers of molasses, sirup, and honey frequently have a compact layer of crystals upon the bottom. Samples taken from the liquid surface and from the crystalline deposits of such products will show the greatest difference in composition. It is therefore necessary to mix thoroughly the contents of a container before sampling. In the laboratory the crystallized sugar in a sample of sirup, molasses, or honey should be redissolved by gentle warming

before beginning the analysis. This is impracticable, however, in sampling these products in bulk from casks or hogsheads, and the most that the sampler can do is to mix the contents as well as possible by shaking and stirring.

The sampling of leaky containers, which allow the escape of liquid but retain all crystallized solids, is a fruitful cause of wide, and often puzzling, discrepancies in analytical results.

### Errors of Analysis Due to Change in Composition of Samples

Owing to the liability of sugar products to change in composition through evaporation or absorption of moisture and through decomposition by the action of enzymes or microörganisms, it is important that analyses be begun as soon as possible after samples are received. It happens, however, in many cases that samples must be sent for a long distance, or stored for a considerable time, before examination can be made; the long storage of products is often necessary, as in the case of reserve samples which are retained for the purpose of confirming an original analysis in the event of doubt or dispute. The sources of error from change in composition of samples will be briefly considered.

**Changes in Composition of Samples through Evaporation or Absorption of Moisture.** — Changes in composition due to this cause are prevented by hermetically sealing the samples in a perfectly tight container. If cans are employed all joints and connections should be soldered; cans of swaged metal, free from seams, are very desirable, but it has not been found possible as yet to manufacture these in large sizes. The covers should fit the cans closely and the space between the two should be sealed by means of melted paraffin or by a band of adhesive tape. In many respects wide-mouth glass bottles or jars are the best containers for samples; the stoppers or corks of these should be sealed by melted paraffin or wax.

In a series of experiments by Stanek * upon the drying out of samples of raw beet sugar in unsealed cans, the average daily evaporation of moisture for 1 month was 0.0115 per cent; when the covers of the cans were sealed with adhesive tape (leucoplast) the average daily evaporation for 1 month was reduced to 0.0006 per cent. This loss from evaporation is of course not evenly distributed, but is greatest during the first few days. Samples of raw cane sugar kept in covered but unsealed cans frequently show a daily increase in polarization, through loss of moisture, of from 0.05 to 0.10 sugar degrees during the first days of storage.

* Z. Zuckerind. Böhmen, **34**, 155.

**Changes in Composition of Samples through Action of Enzymes.** — Changes in composition due to this cause are frequently noted during the storage of plant substances, such as grains, seeds, fruits, tubers, etc. The change may consist in an inversion of sucrose by action of invertase, in a conversion of starch by action of diastase, in a modification of gums, hemicelluloses, etc., by action of other enzymes, or in a loss of sugars through respiration. It is impossible to preserve untreated plant materials of the above description for any length of time without change in composition, although the rate of change may be greatly retarded by cold storage. Heating the samples before storing will destroy enzymes, but has the disadvantage in some cases of causing inversion or of liquefying and saccharifying starch. Freezing the material may suspend enzyme action for the time, but may on the other hand incite changes of a different character, as in the production of sucrose from starch in frozen potatoes.

When samples of fresh plant materials, which are liable to undergo enzymic decomposition, cannot be analyzed immediately, an effective method of preventing change is to weigh out a quantity of the finely reduced substance and preserve in a stoppered jar or bottle by the addition of alcohol. An excess of alcohol (over 50 per cent) destroys the action of enzymes, and samples thus preserved do not undergo any change in composition after many months' standing.

Changes in composition through enzyme action may also occur in cold-strained honey. It has happened in the author's experience that a bottle of such honey, which contained over 20 per cent sucrose at the time of sampling, contained after 4 months' storage less then 10 per cent; in a second sample of the same honey, which was kept in a warm laboratory during the same period, the sucrose was almost completely inverted. The inversion was probably due to an invertase secreted by the bees. The action of enzymes in such products as honey may be destroyed by heating the sample to a temperature of 80° C.

**Changes in Composition of Samples through Action of Microorganisms.** — The effect of yeasts, moulds, and bacteria in changing the composition of sugar products is well known. While the conditions for the development of microörganisms are most favorable in such dilute media as juices and musts, they may also cause deterioration in such concentrated products as molasses and sugar. The fermentation of such a thick menstruum as molasses, however, is confined entirely to the surface, which, through the attraction of hygroscopic moisture, becomes dilute enough to favor microörganic growth. The same is true of raw sugars; the film of molasses coating the crystals undergoes a

gradual fermentation, with the result that the underlying sucrose is slowly dissolved and inverted.

The changes which may occur as a result of fermentation in stored samples of raw cane sugar may be seen from the following polarizations made by Browne * at the Louisiana Sugar Experiment Station upon several samples of Cuban Centrifugal sugars after keeping 9 months in the can.

TABLE III

*Showing Deterioration of Sugar Samples in Storage*

| Number. | April, 1904. | January, 1905. | Decrease. |
|---|---|---|---|
| | Polarization. | Polarization. | |
| 1 | 96.50 | 95.60 | 0.90 |
| 2 | 96.05 | 95.00 | 1.05 |
| 3 | 95.50 | 93.20 | 2.30 |
| 4 | 94.20 | 91.70 | 2.50 |
| 5 | 97.15 | 94.60 | 2.55 |
| 6 | 93.95 | 91.10 | 2.85 |
| 7 | 94.70 | 91.20 | 3.50 |
| 8 | 95.00 | 91.20 | 3.80 |
| 9 | 95.90 | 91.50 | 4.40 |
| 10 | 96.80 | 90.70 | 6.10 |
| 11 | 96.20 | 89.00 | 7.20 |
| Average | 95.63 | 92.25 | 3.38 |

The preservation of sugars and sugar products against microorganisms by sterilization is not always desirable on account of the changes which the high temperature may produce in the physical and chemical properties of the sample. Sterilization of sugar products in order to be effective must be repeated upon several successive days owing to the extreme resistance of many spores to a single heating.

The preservation of liquid sugar products such as juices, musts, sirups, etc., is sometimes effected by adding 0.05 per cent of formaldehyde solution (40 per cent strength) or 0.02 per cent of mercuric chloride.

The preservation of succulent plant substances, such as pulp of fruits, etc., is best accomplished by treating a weighed portion of the sample with alcohol in a stoppered jar or bottle, in the manner previously described.

Other essentials pertaining to the sampling of sugar-containing materials will be described elsewhere.

* Bull. 91, Louisiana Sugar Expt. Station, p. 103.

# CHAPTER II

## DETERMINATION OF MOISTURE IN SUGARS AND SUGAR PRODUCTS BY METHODS OF DRYING

The accurate determination of moisture, in some respects the most simple of analytical operations, is frequently one of the most difficult determinations which the sugar chemist is called upon to make. Among the chief difficulties which confront the chemist in determining the moisture content of sugar products by the ordinary methods of drying, may be mentioned: (1) the very hygroscopic nature of many sugar-containing materials and the retention of water by absorption or occlusion; (2) the extreme sensitiveness of some sugars, notably fructose, to decomposition at temperatures between 80° and 100° C., with splitting off of water and other volatile products; (3) the liability of many impure sugar-containing substances to absorb oxygen during drying, with formation of acids and other decomposition products. The moisture determination is further complicated by the fact that many sugars, as maltose, lactose, and raffinose, retain variable amounts of water of crystallization under different conditions of drying, so that the chemist is not always certain — even when no further loss of weight occurs in the oven — as to the exact amount of moisture which may be retained in a hydrated form.

In the following description of processes for determining moisture, methods will be given for a number of typical substances. The first class of methods to be described is intended only for products which are stable at 100° to 110° C. The determination of moisture in cane sugar is taken as an illustration.

### Determination of Moisture in Cane Sugar

Refined sugar, raw beet sugar, and the superior grades of raw cane sugar are dehydrated successfully by drying 2 to 5 gms. of the finely powered sample in a thin layer for 2 to 3 hours in a boiling-water oven and then heating in a special oven for 1 hour at 105° to 110° C. The sugar is cooled in a desiccator, and, after determining the loss in weight, reheated at 105° to 110° C. for another hour. The process is continued until successive heatings cause no further loss.

For weighing out the sugar flat-bottomed aluminum, nickel, or platinum dishes may be used; clipped watch glasses are also convenient. (See Figs. 3 and 4.) With lower-grade sugars, which contain hygroscopic salts and other impurities, the dish should be covered during weighing. For many purposes of dehydration low glass-

Fig. 3

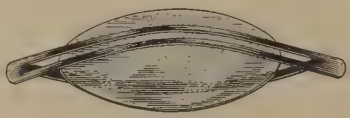


Fig. 4

Fig. 5

Receptacles for drying sugar.

stoppered weighing bottles (Fig. 5) are well suited, and prevent loss of moisture in weighing out the sample and absorption of moisture in weighing the dry residue.

The official method* of the Association of Official Agricultural Chemists for determining moisture in sugars prescribes drying in a hot-water oven for 10 hours. With some sugars, more especially those of large grain, there is danger of occlusion and retention of water, and the last traces of moisture may not be expelled at 98° to 100° C. The method of the International Commission† upon Unification of Methods for Sugar Analysis prescribes in case of normal beet sugars drying at 105° to 110° C.; this temperature is sufficient to expel the last traces of occluded water and is not attended with sufficient decomposition to affect the weight of product. The temperature of drying by this method should not exceed 110° C.

For maintaining a uniform temperature of 105° to 110° C. a glycerin or salt-water bath may be used. The Soxhlet drying oven, shown in Fig. 6, is favored by many for rapid drying. The bath is filled with a salt solution of the desired boiling point, and closed with the condenser *B*. The material is placed in the oven and the door tightly clamped at *A*. Upon lighting a gas flame in the chimney *C* a current of air is generated through the flues at *F*, and, after being heated by the boiling salt solution, passes forward from the back of the drying chamber over the material to be dried. The thermometer *T* indicates the temperature of the drying chamber. By raising the

* Bull. 107 (revised), U. S. Bureau of Chem., p. 64.

† Proceedings, Paris Convention, 1900.

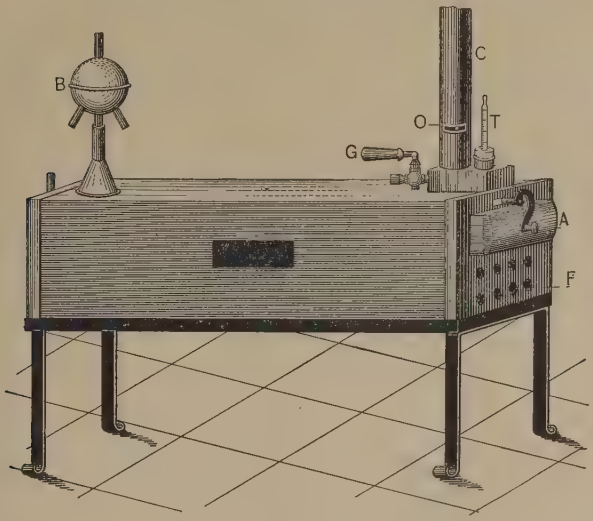



Fig. 6. — Soxhlet drying oven.

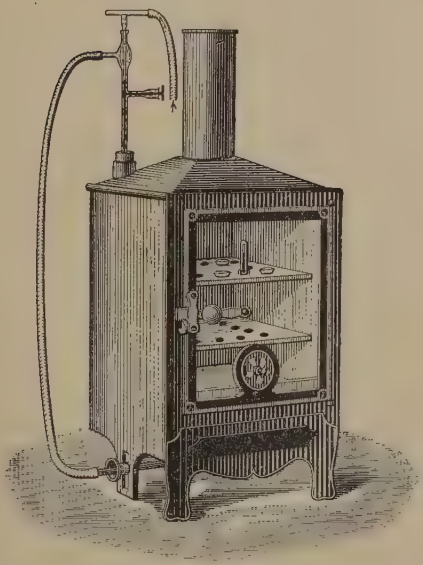


Fig. 7. — Wiesnegg hot-air oven with Reichert gas regulator.

temperature gradually to 100° C. and then to 105° C. for the final dehydration, the time of drying by the Soxhlet oven may be reduced in many cases to less than an hour. A mixture of glycerine and water of the desired boiling point is less liable to corrode the metal of the oven than the salt solution, and is preferred by many for this reason.

In case a hot-air oven is used for drying at 105° to 110° C., the temperature should be governed by means of a gas regulator. A Wiesnegg hot-air oven with porcelain inner chamber and glass door is a very suitable type. Illustration with Reichert gas regulator is shown in Fig. 7. In using hot-air ovens, where considerable variations in temperature are liable to occur through unequal distribution of heat, the exact temperature of drying should be determined by a thermometer placed near the material under examination.

### Determination of Moisture in Sirups, Molasses, Massecuites, etc., when Fructose is Absent or Present only in Traces

For dehydrating sirups, molasses, massecuites, and other sugar-containing substances, which contain but little or no fructose, the method of drying previously described may be used. The material, however, should first be absorbed upon dry sand, pumice stone, or asbestos in order to facilitate the removal of the large excess of water. The following provisional methods* of the Association of Official Agricultural Chemists are recommended for drying the semiliquid products of this class:

*Drying upon Pumice Stone.* — "Prepare pumice stone in two grades of fineness. One of these should pass through a 1-mm. sieve, while the other should be composed of particles too large for a millimeter sieve, but sufficiently small to pass through a sieve having meshes 6 mm. in diameter. Make the determination in flat metallic dishes or in shallow, flat-bottom weighing bottles. Place a layer of the fine pumice stone 3 mm. in thickness over the bottom of the dish and upon this place a layer of the coarse pumice stone from 6 to 10 mm. in thickness. Dry the dish thus prepared and weigh. Dilute the sample with a weighed portion of water in such a manner that the diluted material shall contain from 20 to 30 per cent of dry matter. Weigh into the dish, prepared as described above, such a quantity of the diluted sample as will yield, approximately, 1 gm. of dry matter. Use a weighing bottle provided with a cork through which a pipette passes if this weighing cannot be made with extreme rapidity. Place the dish in

* Bull. 107 (revised), U. S. Bureau of Chem., p. 64.

a water oven and dry to constant weight at the temperature of boiling water, making trial weighings at intervals of 2 hours. In case of materials containing much levulose or other readily decomposable substances, conduct the drying in vacuo at about 70° C."

*Drying upon Quartz Sand.* — "In a flat-bottom dish place 6 to 7 gms. of pure quartz sand and a short stirring rod. Dry thoroughly, cool in a desiccator, and weigh. Then add 3 or 4 gms. of the molasses, mix with the sand, and dry at the temperature of boiling water for from 8 to 10 hours. Stir at intervals of an hour, then cool in a desiccator, and weigh. Stir, heat again in the water oven for an hour, cool, and weigh. Repeat heating and weighing until loss of water in one hour is not greater than 3 mgs.

"Before using, digest the pure quartz sand with strong hydrochloric acid, wash, dry, ignite, and keep in a stoppered bottle."

In order to prevent the occlusion or retention of water in the dried residue, an hour of drying at 105° to 110° C. is advisable as under the determination of moisture in sugar.

**Pellet's Method of Determining Moisture.*** — In a method of drying considerably employed in France, Pellet nickel capsules, 85 mm.

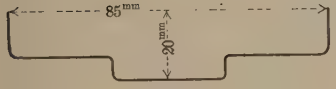



Fig. 8

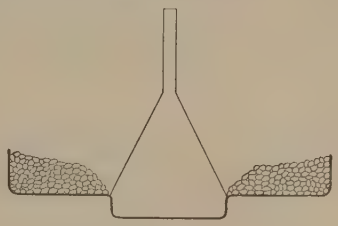


Fig. 9

Pellet capsule for drying liquid sugar products.

wide and 20 mm. deep, are used. The capsule has a circular depression in the center as shown in Fig. 8. Each capsule is provided with a cover having a small notch at the edge for the passage of a small stirring rod.

The raised border of the capsule is filled with fine particles (about 1 mm. diameter) of freshly ignited pumice stone, employing an inverted funnel as shown in Fig. 9. The funnel is then removed, the cover and stirring rod put in place, and the capsule weighed. Three grams of the substance to be dried are then weighed in the central depression of the capsule; 5 c.c. of hot distilled water are then added, and after

* Fribourg's "Analyse chimique" (1907), pp. 90–94.

stirring to dissolve all soluble matter, the capsule is slightly inclined on different sides to permit absorption of the solution by the pumice stone. The process is repeated with 3 c.c. more of hot water and then with 2 c.c. The contents of the capsule are then spread evenly over the entire bottom and dried in any suitable oven at a final temperature of 102° to 105° C.

In case of products containing even traces of free acid, a drop or two of strong ammonia is added. The excess of ammonia is expelled and the amount retained in the combined form is usually too small to be regarded. If the free acid is not neutralized, inversion of sucrose may result, with the introduction of a considerable error in the determination.

### Determination of Moisture in Products Which Contain Fructose

Owing to the susceptibility of fructose to decomposition in presence of water at temperatures much above 70° C., the methods previously described are not applicable to the determination of moisture in such products as honey, sugar-cane molasses, jams, fruit products, and other similar substances. The error which may result from this source may be seen from the following experiment by Carr and Sanborn upon dehydrating a solution containing 17.75 per cent of fructose. The solution was dried upon pumice stone in flat-bottomed dishes at 100° C. in air.

| Hours of drying. | Per cent of solids. |
|---|---|
| 1 | 19.02 |
| 2 | 18.53 |
| 3 | 18.57 |
| 4 | 18.16 |
| 5 | 17.42 |
| 6 | 17.34 |
| 8 | 16 90 |

It is seen that the per cent of solids after 5 hours' drying is lower than the actual amount of fructose taken.

**Methods of Drying in Vacuum.** — The susceptibility of many sugar products to decomposition at 100° C. in the air induced Scheibler in 1876 to propose drying in vacuum. Weisberg* in 1894, and Carr and Sanborn† in 1895, further emphasized the necessity of vacuum drying; and at present dehydration at low temperature under reduced

* Bull. assoc. chim. sucr. dist., **11**, 524.

† Bull. 47, U. S. Bureau of Chem., pp. 134–151.

atmospheric pressure is the only recognized method for the accurate determination of moisture in fructose-containing materials.

*Carr and Sanborn's Method.* — Many methods have been devised for drying sugar solutions in vacuum. The following process is the one described by Carr and Sanborn,* who have employed their method successfully upon the widest range of materials, such as fructose solutions, honey, molasses, sorghum and maize juices, etc.

"Select clean, fine-grained pumice stone and divide into fragments the size of No. 4 shot. Pass the dust through a 40-mesh sieve and treat separately from the larger particles. Digest hot with 2 per cent sulphuric acid and wash until the last trace of acid disappears from the wash water. Owing to the ready subsidence of the material, the washing may be accomplished rapidly by decantation. After complete washing, place the material, wet, in a Hessian crucible, and bring to redness in a monitor or other convenient furnace. When complete expulsion of water is assured, place, hot, in a desiccator, or direct into the drying dishes if desired for use immediately. In loading the dishes place a thin layer of the dust over the bottom of the dish to prevent contact of the material to be dried with the metal; over this layer place the larger particles, nearly filling the dish. If the stone has been well washed with the acid, no harm may result from placing the dish and stone over the flame for a moment before placing in the desiccator preparatory to weighing.

" If the material to be dried is dense, dilute until the specific gravity is in the neighborhood of 1.08 by dissolving a weighed quantity in a weighed quantity of water. (Alcohol may be substituted in material not precipitable thereby.) Of this, 2 to 3 gms. may be distributed over the stone in a dish, the area of which is in the neighborhood of 3 sq. in., or 1 gm. for each square inch of area. Distribute this material uniformly over the stone by means of a pipette weighing bottle (weighing direct upon the stone will not answer), ascertaining the weight taken by difference.

" Place the dishes in a vacuum oven, in which may be maintained a pressure of not more than 5 in. mercury, absolute. The form of oven is not material so long as the moisture escapes freely by passing a slow current of air (dried) beneath the shelf supporting the dishes. The temperature must be maintained at 70° C. and the vacuum at 25 in.

"All weighings must be taken when the dish is covered by a ground plate, and the open dish must not be exposed to the air longer than

* Bull. 47, U. S. Bureau of Chem., pp. 134–151.

absolutely necessary. Weighings should be made at intervals of 2 or 3 hours."

The following triplicate series of experiments were made by Carr and Sanborn upon a solution containing 17.10 per cent fructose. The solution was dried on pumice stone in flat-bottomed dishes at 70° C. under a vacuum of 25 in.

| Hours. | Number 1. | Number 2. | Number 3. | Means. |
|---|---|---|---|---|
| | Per cent. | Per cent. | Per cent. | Per cent. |
| 4 | 17.12 | 17.09 | 17.06 | 17.09 |
| 8 | 17.11 | 17.09 | 17.08 | 17.09 |
| 12 | 17.06 | 17.05 | 17.06 | 17.06 |
| 17 | 17.09 | 17.07 | 17.07 | 17.08 |

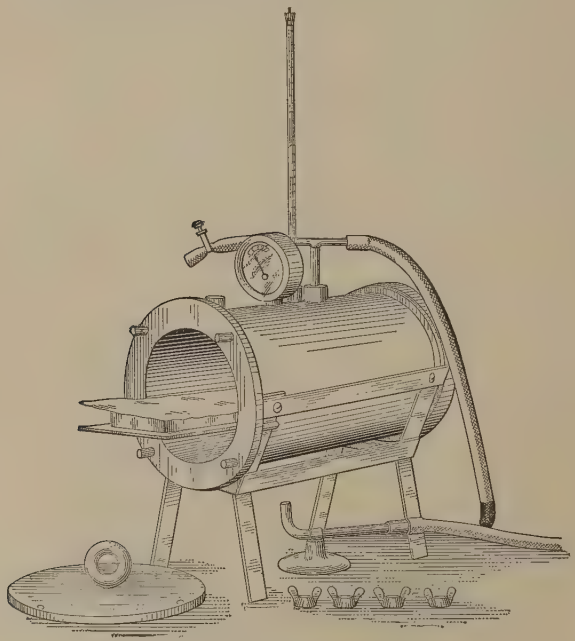


Fig. 10. — Carr vacuum oven.

It is seen that constancy in weight is secured after 4 hours, and that no further appreciable loss takes place even after 17 hours' drying.

An illustration of the Carr vacuum oven is shown in Fig. 10. The oven is provided with openings for attachment of manometer, insertion

of thermometer, and for inlet and exit of air. A gas drier containing concentrated sulphuric acid may be used for removing moisture from the slow current of entering air. The detachable plate at the end of the oven is provided with a rubber gasket and is fastened into position by four screws which secure a perfectly air-tight joint.

*Browne's Method of Vacuum Drying.* — When one of the specially constructed types of vacuum drying oven is not available, the author has found the following arrangement (Fig. 11), which is easily constructed from ordinary laboratory materials, to be perfectly efficient.

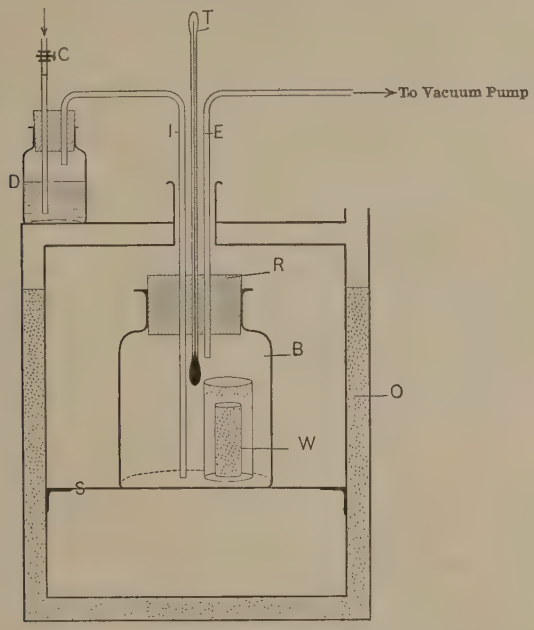



Fig. 11. — Browne's method of vacuum drying.

The vacuum chamber consists of a large-mouth bottle (*B*) of heavy glass, which is supported by the shelf (*S*) of an ordinary water oven (*O*). The mouth of the bottle is closed by a tight-fitting rubber stopper (*R*) whose 3 holes permit the insertion, through the top opening of the oven, of the tubes *I* and *E* and the thermometer *T*. The bottle is easily fitted, and detached from the stopper by first withdrawing the shelf, the latter being shoved into position again when the bottle is in place. The current of air entering by tube *I* to the bottom of the

vacuum bottle is controlled by a clamp pinchcock (*C*) and freed of moisture by a gas drier (*D*). The exit air from the vacuum bottle passes by the tube *E* to the vacuum pump or aspirator.

For absorbing the sugar-containing liquid, asbestos in perforated brass or copper tubes is used. The tubes measure 9 cm. long by 2 cm. in diameter, and are nearly filled with freshly ignited asbestos, the latter being tightly packed with a rod against the sides in the upper half of the tube, so as to leave a central cavity.

Each tube thus prepared is placed in a glass-stoppered weighing bottle of sufficient size, and the whole weighed. About 5 c.c. of the liquid to be analyzed are then delivered from a pipette into the cavity in the asbestos, the object of the cavity being to secure a rapid absorption and even distribution of the liquid through the asbestos. The weighing bottle is then immediately stoppered and reweighed, the increase in weight being the amount of substance taken. After removing the stopper the weighing bottle with tube is placed in the vacuum bottle, as shown by *W* in the diagram, and the temperature raised to 70° C. During the first few hours of drying a brisk current of air is drawn through the vacuum bottle in order to remove the large excess of moisture first given off. In the last stages of the drying the air current is decreased and the vacuum kept at about 25 in. At the end of a few hours the weighing bottle is removed, allowed to cool in a desiccator, and then restoppered and weighed. The bottle is then redried for a second short period to determine if all moisture has been expelled.

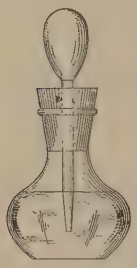


Fig. 12. — Bottle for weighing sugar solutions.

In the weighing out of juices, sirups, sugar solutions, etc., for absorption upon pumice stone, sand, or asbestos, a small flask provided with a stopper and a rubber-bulbed pipette or medicine dropper will be found convenient (Fig. 12). The bottle is filled about two-thirds full with the sugar solution, which should not contain over 25 per cent solids, and then closed with the stopper and pipette. After weighing the bottle and contents, about 5 c.c. of liquid are conveyed by means of the bulb pipette to the absorbent material, and the flask restoppered and weighed. The difference in weight is the amount of sample taken. Honeys, molasses, jellies, and other water-soluble substances of high density should be diluted before this method is employed, by dissolving a weighed amount of substance in a weighed amount of water.

The above method of weighing samples is precluded, however, when

insoluble matter is present, as with jams, sauces, and similar products. In such cases a weighed amount of the well-mixed sample is stirred with a little water until all soluble matter is dissolved and then completely transferred to the absorbent material in the drying dish with help of a fine jet of water. The Pellet method of drying is especially convenient for products of this class.

### Determination of Moisture in Sugar Materials Which Contain Water of Hydration

Difficulty is sometimes experienced in dehydrating sugars such as glucose, lactose, maltose, and raffinose, which crystallize with one or more molecules of water of crystallization. The principal precaution to be observed in drying such sugars is not to raise the temperature in the first stages of the process above the melting point of the hydrate, otherwise the sugar will liquefy to a thick viscous mass from which it is difficult to expel the last traces of water without decomposition.

For drying glucose hydrate, $C_6H_{12}O_6 + H_2O$, the sugar is spread in a thin layer and gently warmed at 50° to 60° C. for several hours, when most of the water will be removed without melting of the crystals. The sugar is then gradually heated to about 105° C., when the last traces of water will be expelled, with no evidence of liquefaction.

For drying raffinose hydrate, $C_{18}H_{32}O_{16} + 5\ H_2O$, the finely powdered sugar is first warmed to 80° C. for several hours and then the temperature gradually raised to about 105° C. The preliminary drying may be hastened greatly by heating the sugar in a vacuum oven.

Maltose hydrate, $C_{12}H_{22}O_{11} + H_2O$, gives off its water very incompletely at 100° C. under atmospheric pressure, and vacuum dehydration is necessary. The sugar is gently heated under a strong vacuum at 90° to 95° C., and then after a few hours the temperature is raised to between 100° and 105° C.

Lactose hydrate, $C_{12}H_{22}O_{11} + H_2O$, retains its water of crystallization unchanged at 100° C. under atmospheric pressure. It is therefore customary in analytical work to estimate lactose as the hydrate. Lactose may be dehydrated, however, by gently heating the finely pulverized sugar in a strong vacuum to a temperature of 125° to 130° C.

The method of drying devised by Lobry de Bruyn and van Laent,* and used by Brown, Morris, and Millar,† and also by Walker,‡ is to weigh the finely powdered sugar in a small flask and connect the latter

* Rec. trav. chim. Pays-Bas, **13,** 218.
† J. Chem. Soc. Trans., **71,** 76.
‡ J. Am. Chem. Soc., **29,** 541.

by a T tube to a bottle containing phosphorus pentoxide, $P_2O_5$, as a dehydrating agent. The open branch of the T tube is connected with a strong vacuum; the flask containing the sugar is then placed in an oil bath and the temperature gently raised to the point desired. Walker found that lactose under these conditions, after heating 1 hour at 80° C. and then 1 hour at 130° C., remained perfectly white, but upon heating to 140° C. the sugar became tinged with brown, showing signs of decomposition.

The method of Lobry de Bruyn and van Laent has also been successfully employed by Rolfe and Faxon * for determining the total carbohydrates in acid-hydrolyzed starch products. In the modified apparatus of Rolfe and Faxon the T tube is provided with a three-way stop-cock, which allows the great excess of water first given off to be removed without coming in contact with the phosphorus pentoxide.

* J. Am. Chem. Soc., **19**, 698.

# CHAPTER III

## DENSIMETRIC METHODS OF ANALYSIS

THE quantity of matter in a unit volume of substance is called the absolute density of that substance. If $m$ be the mass and $V$ the volume of a given substance, its absolute density $D$ will be $D = \frac{m}{V}$. The ratio between the masses of equal volumes of a substance and of some standard material is the relative density of that substance. Since, however, the masses of two bodies at any one place are proportional to their weights, the relative density $S$ of a given substance may be expressed $S = \frac{w}{W}$, where $w$ and $W$ are the weights respectively of equal volumes of the substance and standard material. Relative density is commonly known as specific gravity, and, since the standard substance of comparison is nearly always water, specific gravity is commonly defined as a number indicating how much heavier a substance or solution is than an equal volume of water.

The determination of specific gravity is one of greatest importance in the analysis of sugars; its great value consists in the fact that solutions of different sugars of equal concentration have very nearly the same specific gravity. The following specific gravities are given for 10 per cent solutions of nine different sugars at 20° C. with reference to water at 4° C.: Arabinose 1.0379, glucose 1.0381, fructose 1.0385, galactose 1.0379, sorbose 1.0381, sucrose 1.0381, maltose 1.0386, lactose 1.0376, raffinose 1.0375. It will be noted that the specific gravity of each sugar solution is but little removed from the average 1.0380, which is almost the same as that of sucrose. It is possible, therefore, by means of specific gravity tables established for solutions of pure sucrose to determine very closely the percentage of dissolved substance for any sugar or mixture of sugars in aqueous solution.

**Units of Volume.** The unit of volume universally employed in sugar analysis is the cubic centimeter. This unit is differently defined and the chemist must distinguish carefully between (1) the metric or true cubic centimeter, (2) the Mohr cubic centimeter, and (3) the reputed cubic centimeter.

*The Metric Cubic Centimeter* is defined as the volume occupied by one gram of water weighed in vacuo at 4° C., the temperature of maximum density ($D = 1.000000$). At 20° C. the metric or true cubic centimeter is equivalent to the volume occupied by 0.998234 gram of water weighed in vacuo, or 0.997174 gram of water weighed in air with brass weights.

*The Mohr Cubic Centimeter* is defined as the volume occupied by one gram of water weighed in air with brass weights at 17.5° C. One Mohr cubic centimeter, as thus defined, is equivalent to 1.00234 metric cubic centimeters.

*The Reputed Cubic Centimeter*, a term introduced by Brown, Morris, and Millar,* is defined as the volume at 15.5° C. of one gram of water weighed in air with brass weights. One reputed cubic centimeter, as thus defined, is equivalent to 1.00198 metric cubic centimeters.

The true or metric cubic centimeter was adopted as the standard unit of volume by the International Commission for Uniform Methods of Sugar Analysis at its meeting in Paris, 1900.

### Specific Gravity Tables for Sugar Solutions

Various tables have been established by different observers which give the specific gravity (sp. gr.) of cane-sugar solutions for different concentrations. These tables are expressed in several ways; they vary according to the temperature which is selected for the determination, 15° C., 17.5° C., or 20° C. being usually taken, and also as to whether the weight of water at 4° C. (true specific gravity) is used for comparison, or water at 15° C., 17.5° C., and 20° C. (relative specific gravity). In expressing specific gravity it is customary to indicate the system employed by writing the temperature of the solution above that of the water; thus, $\frac{15°}{4°}$, $\frac{20°}{4°}$, $\frac{17.5°}{17.5°}$, $\frac{20°}{20°}$, etc.

In Table IV the specific gravities of sucrose solutions at several concentrations are given according to the calculations of different authorities.

Various formulæ have been worked out for expressing the relationship between the specific gravity and percentage by weight of dissolved sucrose. Gerlach for specific gravity $\frac{17.5°}{17.5°}$ has expressed the relationship by the equation

$$y = 1 + 0.00386571327\,x + 0.00001414091906\,x^2 + 0.0000000328794657176\,x^3,$$

in which $y$ is the specific gravity and $x$ the per cent of sugar.

* J. Chem. Soc., **71,** 78 (1897).

Scheibler has recalculated Gerlach's equation for sugar solutions of different temperatures with the following results:

| Temperature. | |
|---|---|
| 0° | $y = 1 + 0.003976844\,x + 0.0000142764\,x^2 + 0.000000029120\,x^3$ |
| 10 | $y = 1 + 0.003915138\,x + 0.0000139524\,x^2 + 0.000000032728\,x^3$ |
| 15 | $y = 1 + 0.003884496\,x + 0.0000139399\,x^2 + 0.000000033806\,x^3$ |
| 20 | $y = 1 + 0.003844136\,x + 0.0000144092\,x^2 + 0.000000030912\,x^3$ |
| 30 | $y = 1 + 0.003796428\,x + 0.0000145456\,x^2 + 0.000000030664\,x^3$ |
| 40 | $y = 1 + 0.003764028\,x + 0.0000143700\,x^2 + 0.000000035192\,x^3$ |
| 50 | $y = 1 + 0.003722992\,x + 0.0000148088\,x^2 + 0.000000032440\,x^3$ |
| 60 | $y = 1 + 0.003683112\,x + 0.0000155904\,x^2 + 0.000000026368\,x^3$ |

TABLE IV

*Specific Gravity of Sucrose Solutions by Different Authorities*

| Sucrose, per cent by weight. | Balling-Brix, $d\,\frac{17.5°}{17.5°}$ C. | Gerlach, $d\,\frac{17.5°}{17.5°}$ C. | Gerlach-Scheibler, $d\,\frac{15°}{15°}$ C. | German Imperial Commission. $d\,\frac{15°}{15°}$ C. | German Imperial Commission. $d\,\frac{20°}{4°}$ C. |
|---|---|---|---|---|---|
| 0 | 1.00000 | 1.00000 | 1.00000 | 1.00000 | 0.99823 |
| 5 | 1.01970 | 1.01969 | 1.01978 | 1.01973 | 1.01785 |
| 10 | 1.04014 | 1.04010 | 1.04027 | 1.04016 | 1.03814 |
| 15 | 1.06133 | 1.06128 | 1.06152 | 1.06134 | 1.05917 |
| 20 | 1.08329 | 1.08323 | 1.08354 | 1.08328 | 1.08096 |
| 25 | 1.10607 | 1.10600 | 1.10635 | 1.10604 | 1.10356 |
| 30 | 1.12967 | 1.12959 | 1.12999 | 1.12962 | 1.12698 |
| 35 | 1.15411 | 1.15403 | 1.15448 | 1.15407 | 1.15128 |
| 40 | 1.17943 | 1.17936 | 1.17985 | 1.17940 | 1.17645 |
| 45 | 1.20565 | 1.20559 | 1.20611 | 1.20565 | 1.20254 |
| 50 | 1.23278 | 1.23275 | 1.23330 | 1.23281 | 1.22957 |
| 55 | 1.26086 | 1.26086 | 1.26144 | 1.26091 | 1.25754 |
| 60 | 1.28989 | 1.28995 | 1.29056 | 1.28997 | 1.28646 |
| 65 | 1.31989 | 1.32005 | 1.32067 | 1.31997 | 1.31633 |
| 70 | 1.35088 | 1.35117 | 1.35182 | 1.35094 | 1.34717 |
| 75 | 1.38287 | 1.38334 | 1.38401 | 1.38286 | 1.37897 |

One of the best-known tables for the specific gravity of sugar solutions is that of Balling* $\left(\frac{17.5°}{17.5°}\right)$, published in 1854, and which served as a basis for the better-known and more complete table of Brix, whose name is now almost universally given to the percentages of sugar or dissolved solids (degrees Brix) derived by densimetric means. Another well-known table is that of Gerlach† $\left(\frac{17.5°}{17.5°}\right)$, published in 1863-64, and which served as a basis for Scheibler's‡ table calculated to $\frac{15°}{15°}$. The

* Z. Ver. Deut. Zuckerind., **4**, 304.
† Dingler's Polytech. Jour., **172**, 31.
‡ Neue Zeitschrift, **25**, 37, 185.

most recent and most accurately established tables are those of the German Imperial Commission* upon Standards, based upon the determinations of Plato, and published in 1898 and 1900. These tables give the percentages of sucrose for specific gravities at $\frac{15^\circ}{15^\circ}$, $\frac{t^\circ}{15^\circ}$, and $\frac{20^\circ}{4^\circ}$. The $\frac{20^\circ}{4^\circ}$ table, which was established according to the requirements of the Fourth International Congress of Applied Chemistry (Paris, 1900), is given in the Appendix (Table 1).

The specific gravity tables of the German Imperial Commission have since been enlarged by Sidersky,† so as to give the grams of sugar for 100 gms., and also for 100 c.c., of solution for $\frac{t^\circ}{4^\circ}$ and $\frac{t^\circ}{15^\circ}$ between 10° and 30° C. and for concentrations between 0° and 30° Brix. For their limited range Sidersky's tables are the most complete of any which have been compiled.

**Influence of Temperature upon the Specific Gravity of Sugar Solutions.** — With increase of temperature, sugar solutions expand in volume and the specific gravity becomes correspondingly less. The coefficient of cubical expansion of sugar solutions varies according to concentration. Josse and Remy‡ give the following coefficients for different sugar solutions between 15° and 25° C.:

TABLE V

*Coefficients of Cubical Expansion for Sugar Solutions*

| *d* 15° C. | *d* 25° C. | Concentration. | Coefficient. |
|---|---|---|---|
| 1.02425 | 1.02211 | 6.32 | 0.0002052 |
| 1.05100 | 1.04365 | 12.75 | 0.0002100 |
| 1.10025 | 1.09744 | 23.88 | 0.0002250 |
| 1.14782 | 1.14452 | 33.71 | 0.0002574 |
| 1.19875 | 1.19500 | 43.81 | 0.0002896 |
| 1.25110 | 1.24718 | 53.37 | 0.0003153 |
| 1.30384 | 1.29962 | 62.39 | 0.0003262 |
| 1.33025 | 1.32591 | 66.74 | 0.0003289 |

The mean coefficient of expansion ($\gamma$) of a solution containing $p$ per cent of sucrose for temperatures between 10° and 27° C. can be found by Schönrock's§ formula with a probable error of only ± 0.000006.

$$\gamma = 0.000291 + 0.0000037\,(p - 23.7) + 0.0000066\,(t - 20) - 0.00000019\,(p - 23.7)\,(t - 20).$$

* Z. ang. Chem. (1898), 774; Z. Ver. Deut. Zuckerind., **50,** 982 to 1079.

† "Les Densités des Solutions sucrées à différentes Températures," Paris, 1908.

‡ Bull. assoc. chim. sucr. dist., **19,** 302.

§ Z. Ver. Deut. Zuckerind., **50,** 419.

Knowing the value of $\gamma$, the specific gravity $dt$ at temperature $t$ can be calculated from the specific gravity $dt_0$ at temperature $t_0$ by the equation

$$dt = dt_0 + dt_0 \times \gamma (t_0 - t).$$

In the employment of temperature corrections in densimetric methods of analysis, it is more customary to apply the correction to the percentage of sugar (degrees Brix) rather than to the specific gravity. The correction is to be added in case the temperature is above, and to be subtracted in case the temperature is below, the standard degree of the table (17.5° C. for the old Brix tables and 20° C. for the new tables of the German Commission). Lists of such corrections are affixed to the standard tables of specific gravities.*

**Determination of Dissolved Solids by Use of Solution Factors.**— In the investigation of starch-conversion products the percentage of solids in 100 c.c. of solution is frequently calculated from the specific gravity by means of a "solution factor." This method was introduced in 1876 by O'Sullivan.† who found that, when 10 gms. of maltose or dextrin were dissolved at 60° F. (15.5° C.) to 100 c.c., a solution of 1.0385 sp. gr. $\left(\frac{15.5°}{15.5°}\right)$ was obtained. Assuming that the percentage of dissolved substance is always proportional to the specific gravity of the solution (which is only approximately true), a solution containing 1 gm. of maltose or dextrin in 100 c.c. should have a specific gravity of 1.00385 at 15.5° C. A solution of specific gravity $d$ should contain at 15.5° C. $\frac{1000(d - 1.000)}{3.85}$ gms. of solids.

Brown, Morris, and Millar‡ determined the solution factors of a number of different sugars for a uniform specific gravity of 1.055 $\frac{15.5°}{15.5°}$ with the following results:

TABLE VI

*Solution Factors of Sugars and Starch Conversions*

| | |
|---|---|
| Anhydrous glucose | 3.825 |
| Anhydrous sucrose | 3.859 |
| Anhydrous invert sugar | 3.866 |
| Anhydrous fructose | 3.907 |
| Anhydrous maltose | 3.916 |
| Low starch conversion ($[\alpha]_D = +149.7$) | 3.947 |
| Medium starch conversion ($[\alpha]_D = +173.9$) | 3.985 |
| High starch conversion ($[\alpha]_D = +188.6$) | 4.000 |
| Dextrin | 4.206 |

* Appendix, Tables 2 and 4. † J. Chem. Soc. (1876), 129.

‡ J. Chem. Soc. (1897), **71**, 72.

The solution factors of glucose, fructose, and maltose have recently been determined by Ling, Eynon, and Lane * with practically the same results as Brown, Morris, and Millar.

For ordinary purposes Brown, Morris, and Millar recommend the use of the sucrose factor 3.86. A comparison of the actual grams of sucrose per 100 c.c. of solution with those calculated by means of this solution factor is given in the following table:

TABLE VII.

| $d\ \frac{15.5°}{15.5°}$. | Sucrose in 100 c.c. of solution. | Sucrose by formula, $\frac{1000\,(d-1.0000)}{3.86}$. |
|---|---|---|
| | Grams. | Grams. |
| 1.0039 | 1.00 | 1.01 |
| 1.0193 | 5.00 | 5.00 |
| 1.0386 | 10.00 | 10.00 |
| 1.0578 | 15.00 | 14.97 |
| 1.0770 | 20.00 | 19.95 |
| 1.0959 | 25.00 | 24.84 |
| 1.1149 | 30.00 | 29.76 |

It is seen that the employment of solution factors, while sufficiently accurate for dilute solutions, is attended with considerable error upon liquids of high concentration. The factor 3.86 is not exactly the same for all sugars, so that this method of estimating solids is only useful for approximate purposes.

If the sugar solution be reduced to a uniform specific gravity of about 1.05 and a correction be made for the true density factor, the constant 3.86 can be employed without serious error. The correction is made by multiplying the results (percentages, specific rotation, reducing power, etc.) obtained by using the factor 3.86 by the value $\frac{3.86}{F}$, in which $F$ is the true solution factor, according to Table VI, of the sugar in question.

**Contraction in Volume of Sucrose and Water Mixtures.** — A phenomenon, which has a most important bearing upon the specific gravity of solutions of sugars and other substances, is that of contraction. If a definite quantity of sucrose, for example, be dissolved in a definite quantity of water, the volume of solution is always less than the sum of the volumes of sucrose and water taken. The same is also true, but to a less extent, of the mixture of sucrose solutions of different concentration and of sucrose solutions with water. The phe-

* J. Soc. Chem. Ind., **28**, 730.

nomenon of contraction in volume during solution of sucrose and water has long been known. It was first observed by Reaumur and Petit le Medecin in 1733, and has been repeatedly studied by many subsequent observers.* The extent of this contraction has been variously estimated. If $x$ is the per cent of dissolved sucrose, the change in volume $v$ according to Brix† is represented by the equation

$$v = 0.0288747\,x - 0.000083613\,x^2 - 0.0000020513\,x^3.$$

Scheibler‡ gives the equation

$$v = 0.0273731\,x - 0.000114939\,x^2 - 0.00000158792\,x^3,$$

according to which the maximum contraction is 0.8937 c.c. for 55.42 gms. sucrose and 44.58 gms. water at 17.5° C. Gerlach gives the maximum contraction as 0.9946 c.c. for 56.25 gms. sucrose and 43.75 gms. water, and Ziegler § as 0.9958 c.c. for 56 gms. sucrose and 44 gms. water.

According to Matthiessen and others,|| the maximum contraction is reached at about 40 per cent sucrose; beyond this there is a decrease until at 60 per cent sucrose the contraction is 0; with concentrations above 60 per cent sucrose there is an expansion in volume. This view of the question is due, according to Plato,¶ to the mistaken idea that dissolved sucrose has the same specific gravity as the crystallized solid ($1.59103\,\frac{15°}{15°}$ for chemically pure powdered sucrose, $1.5892\,\frac{15°}{15°}$ for chemically pure sucrose crystals). If we take Plato's calculated value for the specific gravity of dissolved sucrose in aqueous solution, 1.55626, the following results (Table VIII) are obtained which are in close concordance with those of Gerlach and Ziegler. The apparent change in specific gravity of dissolved sucrose is due to the phenomenon of contraction, for which no satisfactory explanation has as yet been offered.

* In contradiction to the results of all previous experimenters, Olizy (Bull. assoc. chim. sucr. dist., **27,** 60) claims to have demonstrated by numerous experiments that absolutely no contraction takes place during the solution of sucrose in water.

† Z. Ver. Deut. Zuckerind., **4,** 308.

‡ Neue Zeitschrift, **25,** 37.

§ Oest. Ung. Z. Zuckerind., **12,** 760.

|| Lippmann, "Chemie der Zuckerarten," 1081.

¶ Z. Ver. Deut. Zuckerind., **50,** 1098.

TABLE VIII

*Showing Contraction in Volume of Sucrose-Water Mixtures*

| Per cent sucrose. | Contraction of mixture. | |
|---|---|---|
| | For 1 kilo. | For 1 liter. |
| | c.c. | c.c. |
| 0 | 0.0 | 0.0 |
| 5 | 1.5 | 1.5 |
| 10 | 2.9 | 3.0 |
| 15 | 4.2 | 4.5 |
| 20 | 5.4 | 6.0 |
| 25 | 6.5 | 7.4 |
| 30 | 7.5 | 8.7 |
| 35 | 8.4 | 9.9 |
| 40 | 9.1 | 11.0 |
| 45 | 9.7 | 12.0 |
| 50 | 10.1 | 12.8 |
| 55 | 10.3 | 13.4 |
| 60 | 10.3 | 13.7 |
| 65 | 10.0 | 13.7 |
| 70 | 9.6 | 13.4 |
| 75 | 8.8 | 12.6 |
| 80 | 7.7 | 11.5 |
| 85 | 6.2 | 9.8 |
| 90 | 4.6 | 7.5 |
| 95 | 2.4 | 4.3 |
| 100 | 0.0 | 0.0 |

The effect of mixing sucrose solutions and water is shown in the following table which gives the calculated contraction of mixtures of 60 per cent sucrose solutions with water to make 100 gms.

TABLE IX

*Showing Contraction in Volume of a 60 Per Cent Sucrose Solution and Water*

| *A* Solution taken. | *B* Volume of solution, 17.5°. | *C* Water taken. | *D* Volume of water, 17.5°. | *E* Volume before mixing, *B*+*D*. | *F* Volume after mixing. | Contraction (*E*–*F*). |
|---|---|---|---|---|---|---|
| Grams. | c.c. | Grams. | c.c. | c.c. | c.c. | c.c. |
| 0 | 0.000 | 100 | 100.126 | 100.126 | 100.126 | 0.000 |
| 5 | 3.876 | 95 | 95.120 | 98.996 | 98.840 | 0.156 |
| 10 | 7.752 | 90 | 90.113 | 97.865 | 97.682 | 0.183 |
| 20 | 15.504 | 80 | 80.101 | 95.605 | 95.372 | 0.233 |
| 40 | 31.008 | 60 | 60.076 | 91.084 | 90.789 | 0.295 |
| 50 | 38 760 | 50 | 50.063 | 88.823 | 88.521 | 0.301 |
| 60 | 46.512 | 40 | 40.050 | 86.562 | 86.273 | 0.289 |
| 80 | 62 016 | 20 | 20.025 | 82.041 | 81.845 | 0.196 |
| 90 | 69 768 | 10 | 10.013 | 79.781 | 79.670 | 0.111 |
| 95 | 73.644 | 5 | 5.006 | 78.650 | 78.595 | 0.055 |
| 100 | 72.526 | 0 | 0.000 | 72.526 | 72.526 | 0.000 |

**The Specific Gravity of Impure Sugar Solutions.** — While the application of specific gravity tables established for sucrose to the estimation of dissolved substance in solutions of other sugars and carbohydrates is fairly accurate, their use in the case of impure sugar solutions may lead to serious errors, owing to the fact that the percentage of dissolved impurities for the same specific gravity differs from the corresponding percentage of sucrose. The errors resulting from this cause may be seen in Table X, which gives the concentrations of sucrose, tartaric acid, sodium potassium tartrate, and potassium carbonate for different specific gravities. When the specific gravity is determined after dilution with a definite amount of water, as is necessary with very thick sirups, the error in estimation of dissolved substance is still further intensified, owing to the difference in contraction

TABLE X

*Concentrations of Aqueous Solutions of Organic and Inorganic Compounds Compared with Those of Sucrose at 15° C. for the Same Specific Gravity*

| Specific gravity. | Sucrose. | Tartaric acid. | NaK tartrate. | $K_2CO_3$. |
|---|---|---|---|---|
| | Per cent. | Per cent. | Per cent. | Per cent. |
| 1.0039 | 1 | 0.87 | 0.57 | 0.43 |
| 1.0078 | 2 | 1.73 | 1.14 | 0.86 |
| 1.0118 | 3 | 2.62 | 1.71 | 1.29 |
| 1.0157 | 4 | 3.49 | 2.28 | 1.72 |
| 1.0197 | 5 | 4.40 | 2.87 | 2.15 |
| 1.0402 | 10 | 8.67 | 5.87 | 4.40 |
| 1.0833 | 20 | 17.52 | 12.16 | 9.00 |
| 1.1296 | 30 | 26.29 | 18.38 | 13.78 |
| 1.1794 | 40 | 35.33 | 24.73 | 18.72 |
| 1.2328 | 50 | 44.22 | 31.10 | 23.76 |

TABLE XI

*Contraction on Diluting Mixtures of Solutions of Above Substances with Water to Reduce Degrees Brix from* 50 *to* 10. *Solution Taken,* 100 *gms.,* 1.2328 *sp. gr., or* 81.49 *c.c. Specific Gravity after Dilution,* 1.0402. *Temperature* 15° *C.*

| Substance. | Dissolved substance, per cent. | | Water added. | | Volume before mixing. | Actual volume after mixing. | Contraction (*E*–*F*). |
|---|---|---|---|---|---|---|---|
| | Before dilution. *A* | After dilution. *B* | $\left(\frac{100\,A}{B}\right)-100.$ *C* | *D* | *E*=(*D*+81.49) | $F=\frac{(100+C)}{(1.0402)}$ | |
| | | | Grams. | c.c. | c.c. | c.c. | c.c. |
| Sucrose..... | 50.00 | 10.00 | 400.00 | 400.34 | 481.83 | 480.67 | 1.16 |
| Tartaric acid. | 44.22 | 8.67 | 410.04 | 410.38 | 491.87 | 490.32 | 1.55 |
| NaK tartrate. | 31.10 | 5.87 | 429.81 | 430.17 | 511.66 | 509.34 | 2.32 |
| $K_2CO_3$..... | 23.76 | 4.40 | 440.00 | 440.37 | 521.86 | 519.13 | 2.73 |

between sugar and dissolved impurities in aqueous solution. This can be seen by reference to Table X; it is also shown in Table XI, which gives the calculated differences in contraction obtained by diluting solutions of sucrose, tartaric acid, sodium potassium tartrate, and potassium carbonate with water to reduce degrees Brix from 50 to 10.

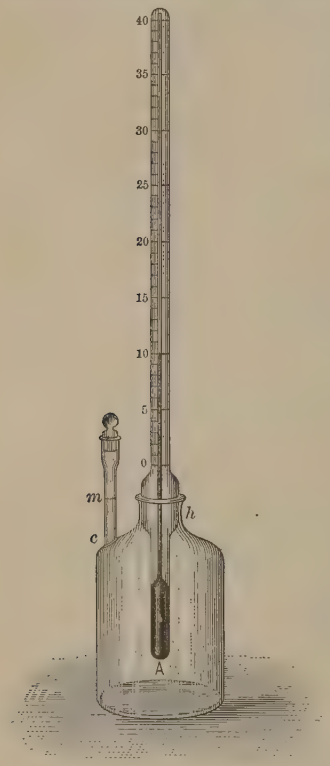



Fig. 13. — Specific gravity bottle with thermometer.

Additional comparisons showing the differences between true dry substance and dry substance as calculated from specific gravity are given for a number of compounds in Table XVII.

### Methods of Determining Specific Gravity of Sugar Solutions

In the estimation of dissolved sugars by means of specific gravity, the temperature of the laboratory is not always the same as that prescribed by the table. It is then necessary either to bring the solution to the required temperature by artificial means or else to apply a fixed correction from a conversion table. The latter method is the more convenient and for ordinary purposes is sufficiently exact; in cases, however, where great accuracy is required the determination must be conducted under absolutely the same temperature conditions as specified in the tables.

**Specific Gravity Bottle or Pycnometer.** — The most accurate method for the determination of specific gravity is the direct comparison of the weights of equal volume of water and sugar solution. In this method some form of specific gravity bottle or pycnometer is used, various types of which are shown in Figs. 13 to 16.

Before using the instrument the pycnometer is calibrated by determining the weight of distilled water which it contains at the temperature of comparison. The bottle is first thoroughly cleaned by means of dilute caustic soda and hydrochloric acid; it is then washed with distilled water and dried in an air bath. In case of pycnometers

constructed with a thermometer stem, the latter should never be warmed beyond the limit of graduation, which is frequently only 40° C., otherwise the expansion of the mercury may break the instrument. After drying and cooling the pycnometer is weighed. The bottle is next filled with distilled water, recently boiled and cooled to expel dissolved air. The temperature adjustment is best effected by filling the bottle with water a degree or so lower than the temperature desired; the stopper is then inserted, taking care to prevent the introduction of air bubbles, and the bottle placed in a bath of water kept exactly at the desired temperature. After about 10 minutes, or as

Fig. 14

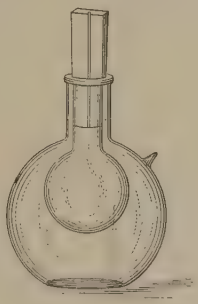


Fig. 15

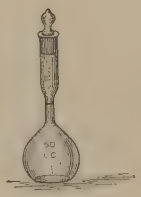


Fig. 16

Types of specific gravity bottles.

soon as the thermometer of the instrument has risen to the right degree, the excess of water, exuding from the stem, or above the graduation mark, is removed with a thin piece of filter paper, the cap is fitted, and the bottle wiped perfectly dry and reweighed. The increase in weight is the water capacity of the bottle at the desired temperature. The process is repeated and the average of several determinations used as a constant in all subsequent work.

The pycnometer, after redrying or rinsing repeatedly with the liquid to be examined, is next filled with the sugar solution (observing the same precautions as to temperature as before) and reweighed. The weight of solution divided by the water capacity of the bottle gives the specific gravity.

Since 20° C. has been adopted as the standard temperature* for

* At the sixth session of the International Commission for Uniform Methods of Sugar Analysis (London, May 31, 1909) it was "voted unanimously to accept a single specific gravity table as standard, at the temperature of 20° C., which is to be based upon the official German table. From this, other tables may be calculated at other temperatures, for instance, at 15° C., 17.5° C., 30° C., etc."

all processes of sugar analysis, it is best to make the determination of specific gravity when possible at this temperature. For the specific gravity $\frac{20^\circ}{4^\circ}$ the value for $\frac{20^\circ}{20^\circ}$ must be multiplied by the density of water at 20° C., or 0.998234.

For very exact work the calculation of specific gravity must be made upon the weights in vacuo, in which case a correction for the density of the air must be introduced. The method of making the calculation is as follows: Let $A$ = apparent weight of pycnometer, $B$ = apparent weight of pycnometer and water at $t^\circ$ C., $C$ = apparent weight of pycnometer and sugar solution at $t^\circ$ C., $d$ = density of water at $t^\circ$ C., and $s$ = density of air at $t^\circ$ C. and the observed atmospheric pressure; then the corrected specific gravity $S$ will be

$$S = d\frac{C-A}{B-A} + s\frac{B-C}{B-A}.$$

If the temperature of the laboratory is much above that of adjustment, the specific gravity bottle and contents must remain at rest until they acquire the surrounding atmospheric temperature, otherwise moisture will condense upon the instrument and interfere with the weighing. It is needless to add that the cap of the bottle must be sufficiently tight to prevent leakage of liquid displaced by expansion through increase of temperature. Pycnometers whose stems are to be filled to mark and hence allow room for expansion, as Fig. 13, are generally to be preferred. For certain kinds of work (as for densities of very dilute sugar solutions) Sidersky* recommends Boot's pycnometer (Fig. 15), which, having a double wall with vacuum, keeps the temperature of the solution constant for a long time.

For highly concentrated sugar solutions, such as molasses, massecuites, or other viscous substances, the method must be somewhat modified, if the specific gravity of the undiluted material is desired. For this purpose a pycnometer with rather wide neck, of the form in Fig. 16, is chosen, and filled nearly to the mark with the hot material to be examined. To remove occluded air bubbles the bottle is placed for a short time in an oil or salt-water bath, the boiling point of which is sufficiently high to keep the material in a liquid condition. After cooling to 20° C. and weighing, the space between the substance and the graduation mark is filled with distilled water and the bottle reweighed. The method of calculation is illustrated by the following example upon a molasses:

* "Les Densités des Solutions sucrées," p. 17.

$A$, water capacity of pycnometer = 50.124 gms.
$B$, weight of molasses = 56.348 gms.
$C$, weight of molasses and water = 66.536 gms.
$C - B$ = weight of water added = 10.188 gms.
$A - (C - B)$ = weight of water occupying space of molasses } = 39.936 gms.

$$\frac{56.348}{39.936} = 1.411 \text{ sp. gr. of molasses.}$$

Reich* has modified the above method by filling the pycnometer to mark directly from a burette divided into 0.05 c.c. and noting the

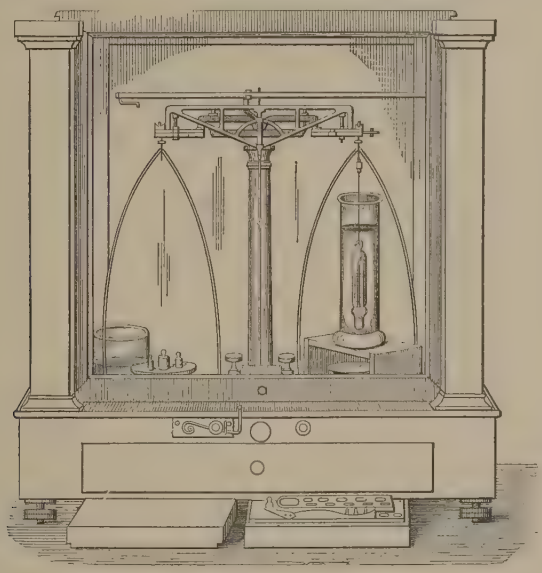


Fig. 17. — Determining specific gravity by means of analytical balance.

volume of water added. If the burette has 50 instead of 0 as the top graduation, the actual cubic centimeters of molasses, etc., in the pycnometer is read off directly when the latter is calibrated to hold exactly 50 c.c. This of course obviates a second weighing of the pycnometer, and, while not as accurate as the method of weighing, is sufficiently close for many purposes.

A second method for determining the specific gravity of sugar solutions is based upon the well-known principle of Archimedes, — that

* Deut. Zuckerind., **34**, 38.

a body immersed in a liquid loses the same weight as that of the volume of liquid displaced. It is therefore only necessary to compare the losses in weight which the same body undergoes in water and in a given solution, in order to determine the specific gravity of the latter. The process may be carried out in a variety of ways; a common method is by means of the analytical balance.

A sinker of heavy glass, or a bulb of glass containing mercury, is attached to a silk thread and weighed first in air, then in distilled water, and finally in the sugar solution. The method of conducting the weighing is shown in Fig. 17.

The method of calculation is shown by the following example:

$A$, weight of sinker in air = 25.345 gms. at 20° C.
$B$, weight of sinker in water = 22.302 gms. at 20° C.
$C$, weight of sinker in sugar solution, = 21.504 gms. at 20° C.

Specific gravity of sugar solution, $S = \frac{A - C}{A - B} = \frac{3.841}{3.043} = 1.2622\ \frac{20°}{20°}$.

To convert to true density with reference to weights in vacuo, the above equation becomes $S\,\frac{t°}{4°} = (d - s)\,\frac{A - C}{A - B} + s$, in which $d$ = density of water at $t°$, and $s$ = density of air at $t°$ and the observed atmospheric pressure.

**Mohr's Specific Gravity Balance.** — The specific gravity balance of Mohr, as improved by Westphal, and hence frequently called the Westphal balance, makes use of the principle of the sinker described in the previous section. The construction and operation of the balance are best understood from Fig. 18. The beam ($AC$) of the balance is pivoted at $B$ and between the pivot and point of suspension ($C$) is divided by notches into 10 equal parts. The distance between each division of the beam is ordinarily made exactly 1 cm. The balance, as usually supplied, has a specially constructed thermometer sinker (Reimann's thermometer body) which by careful grinding of the lower end is made to displace exactly 5 gms. of distilled water at 15° C. The sinker is attached by means of a fine platinum wire to the brass hanger $H$, the combined weight of sinker, wire, and hanger being made to equal exactly 15 gms. Before using, the balance is first adjusted by hanging the sinker from the arm and regulating the screw $S$ until, when the beam is at rest, the pointers of the arm and support at $A$ exactly coincide. If the sinker be now submerged in distilled water at 15° C., it will require 5 gms. at the point of suspension $C$ to restore equilibrium. The standard weight for Reimann's thermometer

body is therefore 5 gms., and in determining the specific gravity of solutions heavier than water this weight must always be hung from the point *C*. To obtain the decimal figures of the specific gravity, weights are added to the notches on the beam until the pointers indicate equilibrium. The first decimal figure is obtained by means of a duplicate 5-gm. weight, which is moved from notch to notch on the beam

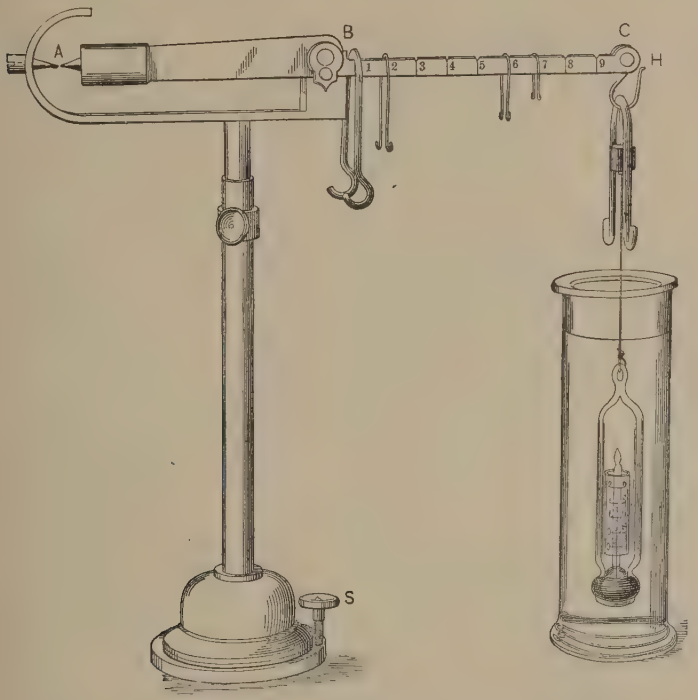



Fig. 18. — Mohr's specific gravity balance (indicating 1.1267 sp. gr.).

until the correct decimal is secured; the second decimal figure is obtained by means of a 0.5-gm. weight, the third decimal figure by a 0.05-gm. weight, and the fourth decimal figure by a 0.005-gm. weight. The specific gravity is then read from the scale divisions of the beam in the order of the diminishing weights. The method of reading is easily understood from Fig. 19.

In using the Westphal balance the temperature of the solution is read from the thermometer of the sinker. In case of turbid or dark-

colored solutions which render the reading of this thermometer difficult or impossible, the temperature is read either by carefully drawing up the thermometer body until the top of the mercury column is visible, or, better, by means of a larger thermometer immersed in the solution. Thermometers and cylinders of special form have been constructed for taking specific gravities, a type of which is shown in Fig. 20.

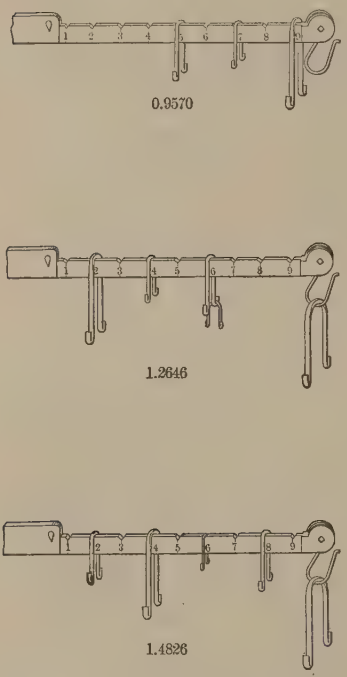



Fig. 19. — Method of reading Westphal balance.

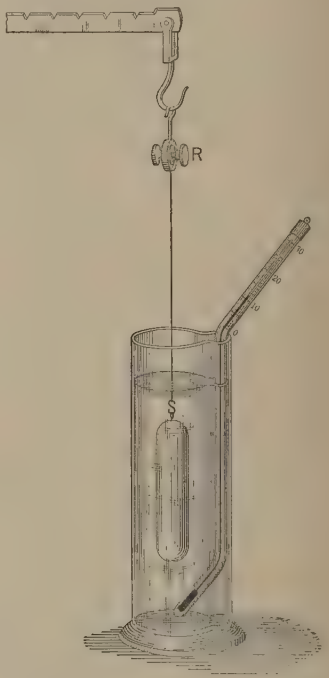



Fig. 20. — Special cylinder and thermometer for Westphal balance.

**Hydrometers.** — A third method of determining the specific gravity of sugar solutions, and the one most commonly employed in technical operations, is by means of the hydrometer. In its usual form (Fig. 21), this instrument consists of a hollow glass body terminating at its lower extremity in a bulb (which can be weighted with mercury or shot) and at its upper extremity in a hollow slender stem, inside of which a paper scale is sealed. If this instrument is allowed to float in a solution, the weight of liquid displaced is equal to the weight of the

floating hydrometer. If placed in solutions of different concentration, the stem will sink to varying depths; that point upon the scale which is level with the surface of the liquid indicates the density or percentage for the given concentration and temperature. It is in this manner that hydrometers are calibrated and standardized.

In actual practice a hydrometer scale is standardized at only a few of its points, the intermediary divisions being determined by interpolation. The method of interpolation will depend upon whether the scale is to indicate specific gravity or direct percentages.

The specific gravity $D$ of a solution is equal to the weight $W$ of the hydrometer divided by the volume $V$ of the part submerged. Then $V = \frac{W}{D}$. If the scale is to be graduated for specific gravity the numerical divisions will proceed in arithmetical progression, such as 1.00; 1.05; 1.10; 1.15; 1.20, etc. The difference between the volumes of the hydrometer for any two scale divisions will give the volume $v$ between these divisions; letting $r$ = half the diameter of the stem, then $\frac{v}{\pi r^2}$ = the distance between the two divisions. The relationship between the stem divisions of a hydrometer weighing 20 gms. and with a cross area of stem ($\pi r^2$) equal to 0.2 sq. cm. can be seen from the following table:

Fig. 21.— Hydrometer.

TABLE XII

*Showing Hydrometer Scale Divided According to Specific Gravity*

| Specific gravity ($D$). | Volume of part submerged $\left(\frac{20}{D}\right)$. | Volume between divisions ($v$). | Distance between divisions $\left(\frac{v}{0.2}\right)$. |
|---|---|---|---|
| | c.c. | c.c. | cm. |
| 1.00 | 20.000 | | |
| | | 0.952 | 4.76 |
| 1.05 | 19.048 | | |
| | | 0.866 | 4.33 |
| 1.10 | 18.182 | | |
| | | 0.791 | 3.96 |
| 1.15 | 17.391 | | |
| | | 0.725 | 3.63 |
| 1.20 | 16.666 | | |
| | | 0.666 | 3.33 |
| 1.25 | 16.000 | | |
| | | 0.615 | 3.08 |
| 1.30 | 15.385 | | |

It will be noted that as the specific gravity increases the distance between the scale divisions decreases. Owing to the great labor involved in the making of calculations and measurements, the division of a hydrometer scale harmonically is accomplished in practice by means of a dividing engine.

In the graduation of a hydrometer scale for indicating direct percentages of sugar, the distance between the scale divisions is much more uniform. The relationship is best seen from the following table, where a hydrometer of 20 gm. weight and 0.2 sq. cm. cross area of stem ($\pi r^2$) was used as before.

TABLE XIII

*Showing Hydrometer Scale Divided According to Sugar Percentage*

| Percentage sugar. | Specific gravity. | Volume of part submerged $\left(\frac{20}{D}\right)$. | Volume between divisions ($v$). | Distance between divisions $\left(\frac{v}{0.2}\right)$. |
|---|---|---|---|---|
| | | c.c. | c.c. | cm. |
| 0.00 | 1.00000 | 20.000 | | |
| | | | 0.772 | 3.86 |
| 10.00 | 1.04014 | 19.228 | | |
| | | | 0.766 | 3.83 |
| 20.00 | 1.08329 | 18.462 | | |
| | | | 0.758 | 3.79 |
| 30.00 | 1.12967 | 17.704 | | |
| | | | 0.747 | 3.74 |
| 40.00 | 1.17943 | 16.957 | | |
| | | | 0.733 | 3.67 |
| 50.00 | 1.23278 | 16.224 | | |
| | | | 0.719 | 3.60 |
| 60.00 | 1.28989 | 15.505 | | |

The maximum difference between the length of the scale divisions in Table XII is 1.68 cm., while for the same range of specific gravity the maximum difference of Table XIII is only 0.26 cm. For a hydrometer graduated to read direct percentages of sugar, it is customary in practice to establish only a few points upon the scale by means of sugar solutions of known concentration, and then divide the intervals between these points into equal subdivisions. While this method is not absolutely accurate, the errors of division are less than the probable errors of observation.

The construction of a hydrometer to read direct percentages of sucrose is first due to Balling. The scale of this instrument, as afterwards recalculated by Brix, constitutes the form at present in most general use. The divisions of the scale are usually called degrees Balling or degrees Brix, as the case may be; the differences between

the two scales are so slight that they have no significance in practical work.

The Brix hydrometer* or spindle is supplied in a variety of forms. For approximate work spindles are used with graduation of 0–30, 30–60, and 60–90, and divided either into 0.5 or 0.2 degree. The forms in most common use, however, have only a range of 10 degrees, 0–10, 10–20, 20–30, 30–40, etc., graduated into 0.1 degree. For greater accuracy a third form of spindle has been made with a range of only 5 degrees, 0–5, 5–10, 10–15, 15–20, etc., and graduated into 0.05 degree. With the help of a spindle for only approximate work, the choice of

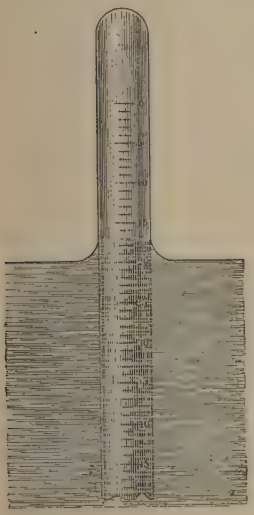


Fig. 22. — Floating Brix spindle.

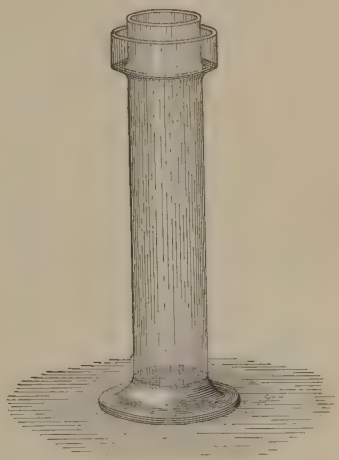


Fig. 23. — Winter's cylinder for taking specific gravity.

the particular hydrometer for the finer reading will be facilitated. The accuracy of the spindle is of course the greater, the smaller the diameter of the stem and the consequently larger interval between the scale divisions.

In determining specific gravity by means of the hydrometer, a tall, narrow cylinder is usually employed for holding the liquid to be examined. The spindle is carefully lowered into the solution in such a

* The term saccharometer, which is sometimes applied to a hydrometer indicating percentages of sucrose, is unfortunate, owing to the confusion with the word saccharimeter, of entirely different meaning.

way that the surface of the stem above the liquid is not moistened. Care should also be exercised that the instrument floats freely and does not touch the bottom or walls of the cylinder. The reading is made by bringing the eye upon a level with the surface of the solution and noting where the border line intersects the scale; the film of liquid drawn up around the stem by capillarity should be disregarded. The reading of the spindle, for example, in Fig. 22, is 20 and not 17. The scale of the hydrometer is read with greater ease when the surface of the liquid is level with the brim of the cylinder. Cylinders of the form designed by Winter (Fig. 23) are convenient for this purpose; any overflow of liquid displaced by the spindle is caught in the circular trough.

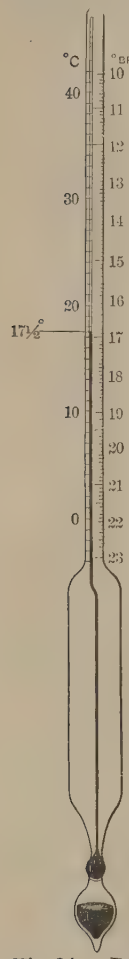



Fig. 24. — Brix spindle with thermometer.

The same attention must be paid to temperature when the hydrometer is employed as in other methods of determining specific gravity. The Brix spindle is calibrated at 17.5° C., and unless the solution be of this temperature a correction must be applied. A table of temperature corrections for degrees of the Brix scale is given in Table 4 of the Appendix; these corrections are to be added to readings made above 17.5° C. and subtracted from those made below.

Brix hydrometers are sometimes fitted with thermometers, a form of which modification is shown in Fig. 24. The advantages of this construction disappear somewhat when working with turbid liquors, which render the reading of the thermometer difficult or impossible. For general purposes the temperature of the solution is best taken by means of an accurately standardized special thermometer.

Volquartz* has constructed a Brix spindle with a correction scale, the mercury of the thermometer in the stem indicating, instead of temperature, the correction necessary to be added to the scale reading. The method of operation may be seen from Fig. 25. The spindle in the illustration indicates 10.0 Brix; the mercury of the thermometer marks 2.7; the reading corrected to 17.5° C. is, then, 10.0 + 2.7 = 12.7 Brix. If the mercury is below the 0 mark (17.5° C.), the correction must be subtracted.

* Z. Ver. Deut. Zuckerind., **46**, 392.

Vosátka* has constructed a Brix spindle with movable scale, which after adjustment to the temperature of the sugar solution gives the true reading directly.

For determining the Brix of dilute sugar solutions, an operation of considerable importance in exhausting filter-press cake ("sweetening off"), a variety of spindles known as "sweet-water" spindles has been constructed. These hydrometers have a large body with a thin stem, so that the readings can be easily made to 0.1 degree. The sweet water as it comes from the filters has usually a temperature of 60° to 80° C., and, to prevent the delay incident to cooling the solution to 17.5° C., sweet-water spindles are often calibrated at high temperatures. One form of such spindle is graduated to read 0 degree Brix in water at 75° C., and 5 Brix in a 5 per cent sugar solution of the same temperature; such a spindle cannot of course be employed at other temperatures, so that its usefulness is somewhat limited.

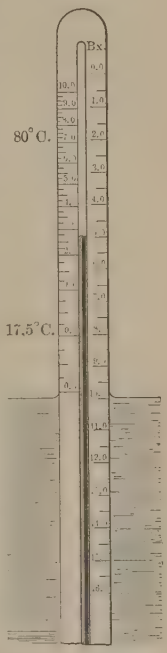


Fig. 25.—Volquartz spindle with temperature correction scale.

Another form of sweet-water spindle (Fig. 26) is graduated from 0 to 5 Brix in the normal way. Below the 0 mark the divisions are continued in the same manner, the result being a double scale with the 0 division in the middle. At 17.5° C. the readings of the upper scale give the true Brix; at temperatures above 17.5° C., sweet waters will read less than the true Brix. At 70° C. a 5 per cent sugar solution reads 0 on the spindle, a 4 per cent solution −1, a 3 per cent solution −2, a 2 per cent solution −3, a 1 per cent solution −4, and pure water −5. The true Brix can be determined for any temperature by means of a correction table; determinations by this instrument can always be controlled by cooling the solution to 17.5° C.

Still another form of sweet-water spindle has been devised by Langen. This spindle (Fig. 27) contains within its body a thermometer graduated from 30° to 70° C. The graduated scale in the stem of Langen's spindle differs from other forms, however, in not giving Brix degrees, but in simply indicating the thermometer reading for each division to which the hydrometer will sink in pure water. If placed, for example, in distilled water of 30° C., the instrument

* Z. Zuckerind. Böhmen, **27**, 689.

will sink to the division 30 on the stem, and in water of 70° C. to the division 70; in other words, the thermometer and scale of the spindle will give the same readings between 30 and 70 when the instrument is floated in distilled water. When the spindle is placed in a sweet water, the reading of thermometer and scale will no longer agree. The spindle necessarily sinks to a lesser depth than in water, and the scale of the stem gives a different reading from that of the thermometer, the difference between the two being proportional to the concentration of solution. In sweetening off, it is only necessary to observe the readings of thermometer and scale; the differences between these decrease as the extraction proceeds, until with the coincidence of the two readings complete exhaustion is indicated.

Fig. 26.— Sweet-water spindle.

Another form of hydrometer which is frequently used in the sugar factory, but to a much less extent in the sugar laboratory, is that of Baumé. This instrument is standardized by means of common salt; the 0 point at the top of the stem is obtained by means of distilled water, and the 15-degree mark by means of a 15 per cent salt solution. The interval between these two divisions is then divided into 15 equal parts, this graduation being extended downwards on the scale as far as desired. Unfortunately, in the early instruments the temperature of the water and the specific gravity of the salt solution were not correctly obtained, so that the values of the Baumé scale divisions have been variously reported by different authorities. The so-called "old" Baumé degrees, as calculated by Brix, are still used in European countries in the commercial analysis of molasses * notwithstanding the fact that Gerlach as long ago as 1870 showed the incorrectness of the formulæ employed by Brix in his calculations.

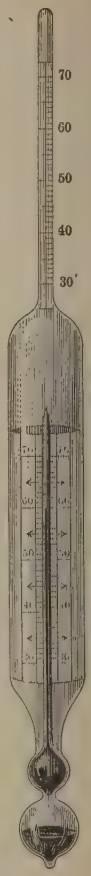



Fig. 27.— Langen's sweet-water spindle.

* Frühling's "Anleitung," p. 74.

Gerlach found as the specific gravity of a 15 per cent salt solution at 17.5° C., 1.11383. The volume of a Baumé spindle up to the 0 mark, in terms of the volume of a single scale division, is then equal to $\frac{1.11383 \times 15}{1.11383 - 1} = 146.78$. The specific gravity $S$ corresponding to any scale division $N$ of the Baumé scale can then be calculated by the formula $S = \frac{146.78}{146.78 - N}$. It is by use of this formula that the so-called "new" Baumé degrees have been determined. The relationship between percentages of sugar, or degrees Brix, specific gravity and the new and old degrees Baumé, is shown in Table 3 in the Appendix.

# CHAPTER IV

## PRINCIPLE AND USES OF THE REFRACTOMETER

A SECOND method of estimating the percentage of sugars in solution is by means of the refractive index. The general applicability of this method, as in the case of specific gravity, depends upon the fact that solutions of all sugars of equal concentration have nearly the same index of refraction.

**Law of Refraction.** — If a beam of light from one medium, such as air, fall at an inclined angle upon the surface of a second medium, such as water, it will be found that the beam upon entering the second medium is bent or deflected from its original course. A good example of this phenomenon, which is called refraction, is the bent appearance of the oar of a boat when seen obliquely under water. There is a general law of refraction for all transparent liquids and solids which may be stated as follows: For two given media and the same ray of light (same wave length), the ratio of the sine of the angle of incidence to the sine of the angle of refraction is always a constant quantity for the same temperature.

In Fig. 28 $m$ and $m'$ are two media; $PP'$ is drawn perpendicular to the dividing surface $FF'$. Let a beam of light pass through $m$ in the direction $LO$; a part of the beam at the point $O$ of the surface is reflected in the direction $OL'$; another part of the beam entering $m'$ is refracted in the direction $OS$. The angle $LOP$ which the falling ray makes with the perpendicular is the angle of incidence, or $i$; the angle $SOP'$ which the refracted ray makes with the perpendicular is the angle of refraction, or $r$. The ratio $\frac{\sin i}{\sin r} = n$ is called the index of refraction. This ratio in Fig. 28 is represented by $\frac{\text{line } ab}{\text{line } cd}$.

The ratio $\frac{\sin i}{\sin r}$ is also that of the velocities of light in the two media. If $v$ is the velocity of light in $m$ and $v'$ the velocity in $m'$, then $n = \frac{\sin i}{\sin r} = \frac{v}{v'}$. If the refracted ray is bent toward the perpendicular as in Fig. 28, the velocity $v'$ is smaller than $v$, and the medium $m'$ is called of greater optical density than $m$. Optical density must not be

confused with material density, since the two expressions do not at all correspond.

If the ray of light in Fig. 28 pass from a denser medium $m'$ into a rarer medium $m$ in the direction $SO$, it will be refracted in $m$ in the direction $OL$. In this case the index of refraction is $\frac{\sin r}{\sin i}$, which is the reciprocal of the index for light passing in the opposite direction. The refractive index varies with the wave length of the light, increasing

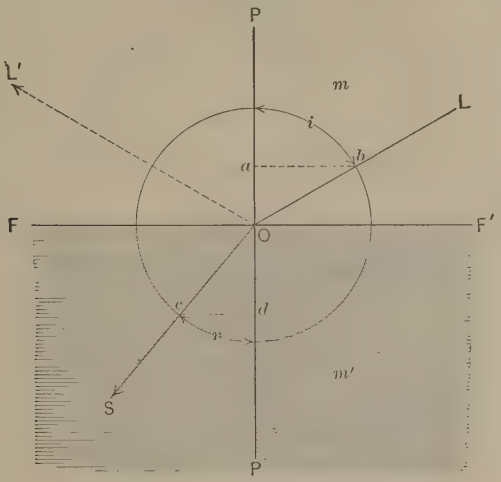



Fig. 28. — Illustrating law of refraction.

from the red towards the violet end of the spectrum. From this it follows that when ordinary light is refracted it is decomposed into light of the different prismatic colors; this unequal refraction for light of different wave lengths is called dispersion.

**Measurement of Refractive Index.** — The refractive index of a solution can be measured in a variety of ways. One of the simplest methods, which is of more value for demonstration than for accuracy, is by means of the refractometer trough. This apparatus, shown in Fig. 29, consists of a semicircular trough, the inner curved surface of which is divided into degrees. The side of the trough corresponding to the diameter of the circle consists of a plate of glass which is made nontransparent, excepting a narrow perpendicular slit at the center $c$. If the trough be filled partly with a solution and a beam of light fall upon the glass, that part of the beam passing through the slit above

the surface of the liquid will mark the angle of incidence and that part passing below the surface will mark the angle of refraction. In the

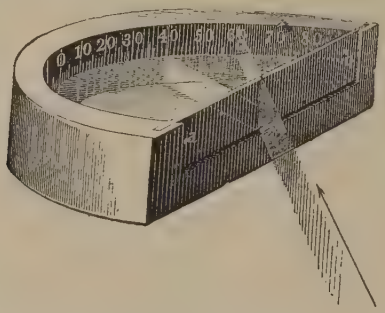



Fig. 29. — Measuring refractive index by refractometer trough.

illustration, where water is used, these angles are 60 degrees and 40 degrees respectively.

$$\frac{\sin 60^\circ}{\sin 40^\circ} = \frac{0.8660}{0.6428} = 1.34 \text{ or } n, \text{ the approximate index of refraction.}$$

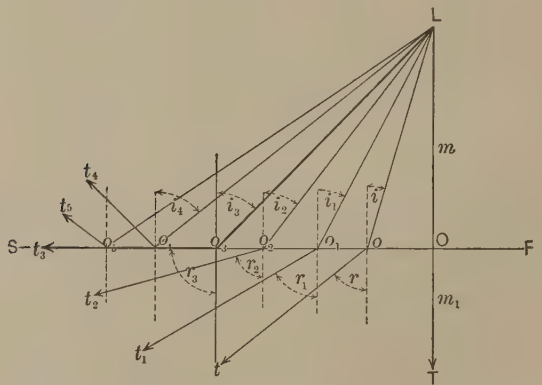



Fig. 30. — Illustrating principle of total reflection.

In the construction of refractometers for more accurate measurements, instrument makers generally employ the method of total reflection. The principle of this method can be understood from Fig. 30.

Let $m$ and $m_1$ be two media, such as glass and water, of which $m$ is

the more optically dense, the dividing surface being $SF$. The beams of light which fall from the source $L$ upon $SF$ at various angles are refracted, in $m_1$ in different directions. The beam $LO \perp SF$ is not refracted and proceeds in the same direction; the beam $Lo$, making the angle of incidence $i$, is refracted in the direction $ot$, making the angle of refraction $r$; in the same way $Lo_1$ is refracted to $o_1t_1$, and $Lo_2$ to $o_2t_2$. As the angle of incidence for the falling beam increases, there finally comes a point at $o_3$ where the refracted ray $o_3t_3$ coincides with the surface $SF$, and the angle of refraction $r_3 = 90$ degrees. If the angle of incidence be increased beyond $i_3$ to $i_4$, the beam which previously was only partly reflected is totally reflected in the direction $t_4$, and there is no refraction in $m_1$. Since $\frac{\sin i_3}{\sin r_3}$, the index for the beam before total reflection, equals $\frac{\sin i_2}{\sin r_2}$, etc., $= \frac{\sin i}{\sin r} = n$, and since $\sin r_3 = 90° = 1$, it is evident that for the border line of total reflection $\sin i = n$. In other words, the sine of the angle of incidence for the border line of total reflection is equal to the refractive index. It is seen from the diagram that total reflection can only take place when light passes into an optically rarer medium.

For absolute measurements the refractive index of a substance is referred to a vacuum. Since, however, the absolute index of air is only 1.000294, refractive indices referred to air are sufficiently exact for most purposes. In the case of three media such as air, glass, and a liquid, if the index from air to glass be $N_{ag}$ and from glass to liquid $N_{gl}$, then the index from air to liquid $N_{al} = N_{ag} \times N_{gl}$. The sine of the angle of incidence for the border line of total reflection between glass and a given liquid, multiplied by the index of refraction between air and glass, will give the index of refraction for the liquid with reference to air.

### Abbe Refractometer

The best general instrument for determining the refractive index of sugar solutions is that of Abbe (Fig. 31). The essential part of the Abbe refractometer consists of two flint-glass prisms $A$ and $B$ of refractive index $n_D = 1.75$, each cemented into a metal mounting. To open the prisms the latter are rotated on their bearings to a horizontal position with the prism $B$ uppermost; the clamp $v$ is then released and prism $B$ swung open on its hinge $C$. A few drops of the solution to be examined are then placed upon the polished inner surface of the fixed prism $A$ next to the telescope, and prism $B$, whose inner surface is

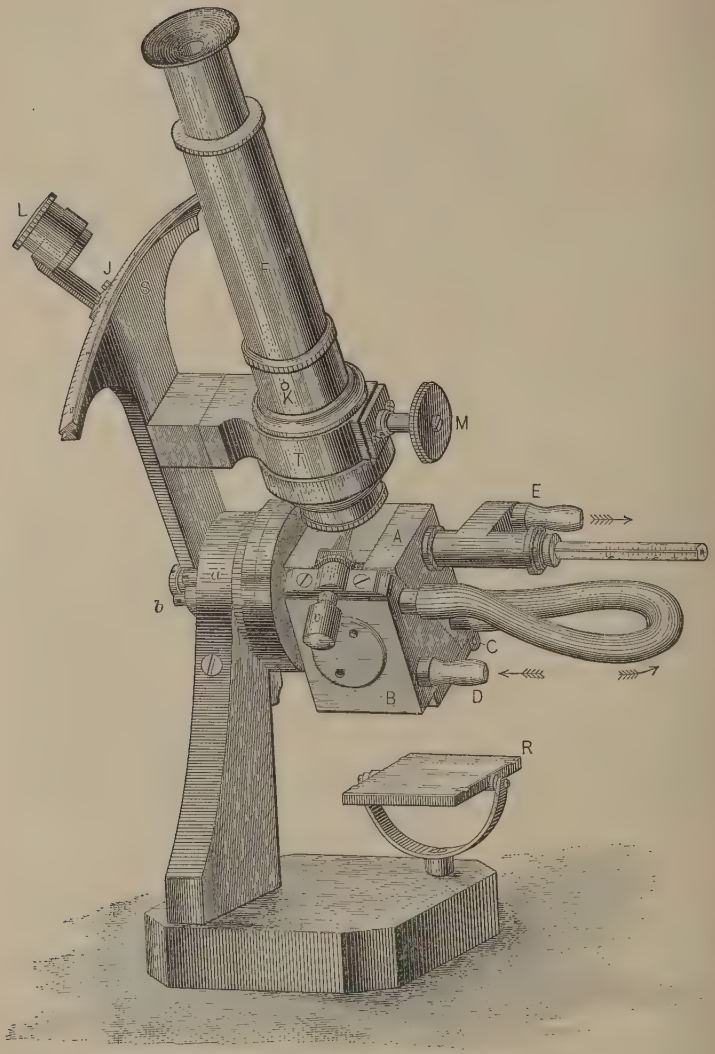



Fig. 31. — Abbe refractometer.

ground, brought slowly back and clamped as before. The instrument is then swung into an upright position and light reflected from the mirror $R$ upon the surface of the lower prism.

In the following diagram (Fig. 32) $FDE$ and $ABC$ are longitudinal sections of the two prisms in an Abbe refractometer between whose hypotenuse surfaces $FE$ and $AB$ (separated by about 1.5 mm.) is the

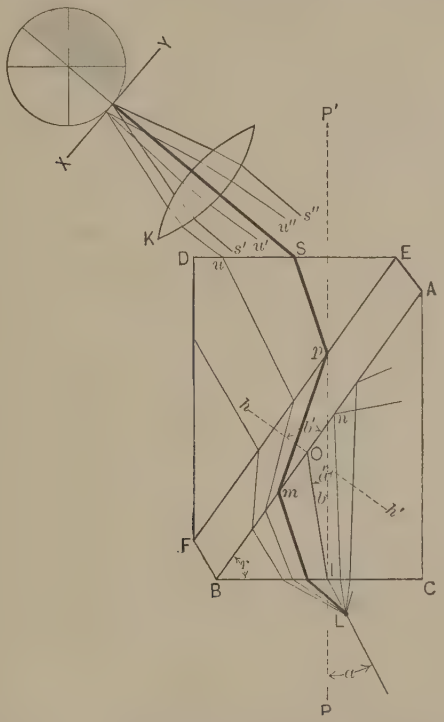



Fig. 32. — Illustrating principle of Abbe refractometer.

film of liquid to be examined. The beams of light passing from $L$ through the lower prism to the surface of the solution $AB$ are refracted or totally reflected, according to the refractive index of the liquid. As shown in the diagram the beams which fall upon the hypotenuse surface $AB$ at a less inclination than the line $IO$ undergo refraction in the liquid, and, passing through the upper prism, the sets of parallel rays $s, s', s'', \ldots, u, u', u'', \ldots$, etc., are condensed by the objective $K$ of the telescope upon the field $XY$. The beams in the

prism parallel to $IO$ are refracted along the surface $BA$ and the beams of greater inclination totally reflected; since these beams do not reach the surface of the upper prism, a part of the field $XY$ remains in shadow.

The telescope of the refractometer ($F$ in Fig. 31) is attached to a sector $S$ and the prisms to a movable arm $J$ (the alidade) which carries a magnifying lens $L$. By moving the alidade until the intersection of the reticule in the telescope field (Fig. 32) cuts the dividing line between the bright and dark portions of the field, the refractive index can be read directly upon the scale of the sector by means of the lens.

The relation between the angles of incidence and refraction of light between air and prism, and prism and liquid, in the Abbe refractometer may be understood from Fig. 32. Let $PP'$ be drawn $\perp$ to the end planes $BC$ and $DE$ of the double prism, and $hh'$ be drawn $\perp$ to the hypotenuse planes $AB$ and $EF$.

Let $a$ = angle of incidence from air and

$b$ = angle of refraction in glass; then

$\frac{\sin a}{\sin b} = n$ for prism, which for the flint glass of the Abbe instrument is about 1.75.

Let $r$ = angle of prism.

$a'$ = angle of incidence in glass upon surface $AB$ and

$b'$ = angle of refraction in liquid = 90 degrees for border line of total reflection.

In $\triangle BOI \angle r + \angle BOI + \angle BIO = 2\,rt.\,\angle$'s;

$\angle BOI + \angle a' + \angle BIO + \angle b = 2\,rt.\,\angle$'s;

whence $r = a' + b$.

By way of illustration the following values are given for $a$, $b$, and $r$, with water as the liquid between the prisms:

$a = 18^\circ\ 32'.$

$b = 10^\circ\ 28'.$

$r = 60^\circ\ 00'.$

$$\frac{\sin a}{\sin b} = \frac{0.3179}{0.1817} = 1.75 = n \text{ for air to prism.}$$

$a' = 60^\circ - 10^\circ\ 28' = 49^\circ\ 32'.$

$$\frac{\sin a'}{\sin b'} = \frac{0.76}{1} = 0.76 = n \text{ for glass of prism to water.}$$

$1.75 \times 0.76 = 1.33 = n$ for air to water.

Each division, therefore, upon the sector of the refractometer representing refractive index is equal to the sine of the angle of incidence in the prism for the border line of total reflection multiplied by the refractive index of the prism. Since total reflection can take place only when light passes from an optically denser to a rarer medium, the capacity of the refractometer is necessarily limited to solutions of smaller refractive index than 1.75.

A second important feature of the Abbe refractometer is the compensator. The function of this is to correct the dispersion which white light undergoes in the double prism. Without the compensator the border line between the light and dark parts of the field, owing to the unequal refraction of light of different wave lengths, assumes the appearance of a band of prismatic colors, which it is impossible to use for purposes of adjustment.

The compensator of the refractometer is placed in the prolongation of the telescope tube between the objective and the double prism. It consists of two similar Amici prisms, such as are used in a direct-vision spectroscope, and which give no divergence for the yellow *D* line of the spectrum (i.e., the emergent *D* rays are parallel with the optical axis). The two prisms are rotated simultaneously in opposite directions by means of the screw head *M* (Fig. 31).

Trapezoidal sections of the two Amici prisms are shown in Fig. 33. Each prism consists of a combination of two crown-glass prisms, with

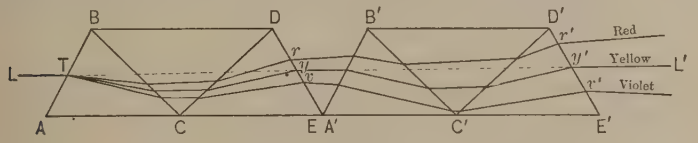



Fig. 33. — Illustrating principles of compensator.

a third right-angled flint-glass prism between them in the manner shown. If a beam of white light *LT* fall upon the surface of the first prism *AB*, it is decomposed into its colored constituents, as shown by the divergent broken lines. In their passage through the prism the red rays are refracted least and emerge at *r*, the yellow rays emerge at *y*, and the violet rays, which are refracted most, emerge at *v*. If the light emerging from the prism *ABDE* now enter a second prism *A′B′D′E′* similarly placed to the first prism (their refracting edges *A* and *A′* being parallel and on the same side of the optical axis *LL′*), the colored rays will emerge from the second prism at the points *r′*, *y′*, and *v′* respectively, the angle of dispersion for any two differently colored rays being twice that for the single prism *ABDE*.

If the two Amici prisms be now rotated in opposite directions around the optical axis $LL'$, the dispersion of the compensator will diminish until, when each prism has rotated 90 degrees (the difference from the previous position being 180 degrees), the dispersions of the two prisms neutralize one another and the dispersion of the compensator is zero. In this position the refracting edges $A$ and $A'$ of the two prisms will again be parallel, but on *opposite* sides of the optical axis $LL'$. If we now imagine the direction of the colored rays through the two prisms to be reversed, we have an exact representation of the work performed in the compensator. The band of colored light from the double prism of the refractometer, passing in the direction $L'L$, emerges at $T$ as a colorless beam, and the bright and dark halves of the field are sharply divided. By rotating the screw head the compensator can be given an equal but opposite dispersion to that of the liquid examined for any value from zero up to twice the dispersion of a single Amici prism.

After setting the compensator to the point where the colored bands disappear, the reading of the scale upon its drum ($T$, Fig. 31) enables one to calculate the dispersion of the liquid examined for the $F$ and $C$ rays of the spectrum, the mean dispersion $n_F - n_C$ (difference in refractive index for the $F$ and $C$ rays) being determined with the help of a special table supplied with the instrument.

Duplicate readings upon the Abbe refractometer with a sharp definition of the border line should agree within two places of the fourth decimal. After each determination the prisms should be cleaned with wet filter paper and then wiped dry with a piece of soft linen.

**Illumination of Abbe Refractometer.** — For illuminating the refractometer ordinary daylight may be used, in which case the instrument should not be placed in the direct light of the sun. Since, however, daylight (especially in winter) is of variable intensity, and upon dark days not strong enough for the examination of deep-colored solutions, it is better on the whole to use artificial light of constant intensity. An incandescent electric lamp or Welsbach gas burner is a most convenient method of illumination. A large sheet of cardboard, placed in front of the instrument so as to shield the light from the upper prism and from the eye of the observer, will protect the field of vision from the disturbing influences of extraneous light and increase to a marked extent the sensibility of adjustment.

**Regulation of Temperature in Abbe Refractometer.** — The refractive index of sugar solutions, as of all other substances, varies with the temperature, the index decreasing as the temperature rises. It is

therefore important in all refractometer work that the temperature be kept constant during the course of observation. In the Abbe refractometer shown in Fig. 31 water of constant temperature is allowed to circulate in the direction of the arrow through the metal casings which surround the prisms; a thermometer screwed into the upper casing indicates the temperature.

**Zeiss Spiral Heater and Water-pressure Regulator.** — A convenient piece of apparatus for controlling the temperature of refractometers is the Zeiss spiral heater and water-pressure regulator. This apparatus shown in Fig. 34 consists of a constant-level reservoir *A* connected by rubber tubing to the water supply and attached to a sliding frame which can be adjusted to different heights. The water passes from the reservoir to the spiral heater, which is placed upon a level below the refractometer. The heater consists of about 12 feet of copper tubing wound in a spiral and inclosed in a metal jacket which is heated by a Bunsen burner. The water flows from the heater upward to the prisms of the refractometer and thence to a constant-level vessel *B*, from which the overflow escapes to a drain. The water, which should not flow too slowly, is first warmed to the approximate temperature by regulating the flame of the burner; the exact adjustment is then made by varying the speed of the flow, which is done by raising or lowering the pressure reservoir on its sliding frame. In this manner the temperature can be maintained for hours within 0.1° C., provided of course that no variations take place in the temperature of the main water supply.

Instead of the Zeiss heater a large insulated heatable tank holding 50 to 100 liters of water may be used.

**Testing the Adjustment of the Abbe Refractometer.** — The adjustment of the Abbe refractometer can be tested by means of liquids or glass test plates of known refractive power. Freshly distilled water free from air ($n_D^{20°} = 1.33298$) is convenient for testing the lower divisions of the sector scale; monobromonaphthalene ($n_D^{20°} = 1.658$) is convenient for testing the upper part of the scale; the latter substance unless freshly prepared usually requires to be redistilled (boiling point 277° C.). The Abbe instrument is supplied with a glass test plate whose index is marked upon the upper ground surface. The method of using the plate, which can be applied to any transparent solid, is that of grazing incidence (explained in detail under the immersion refractometer).

In using the test plate the instrument is reversed as shown in Fig. 35, the double prism spread open, and the polished surface of the plate

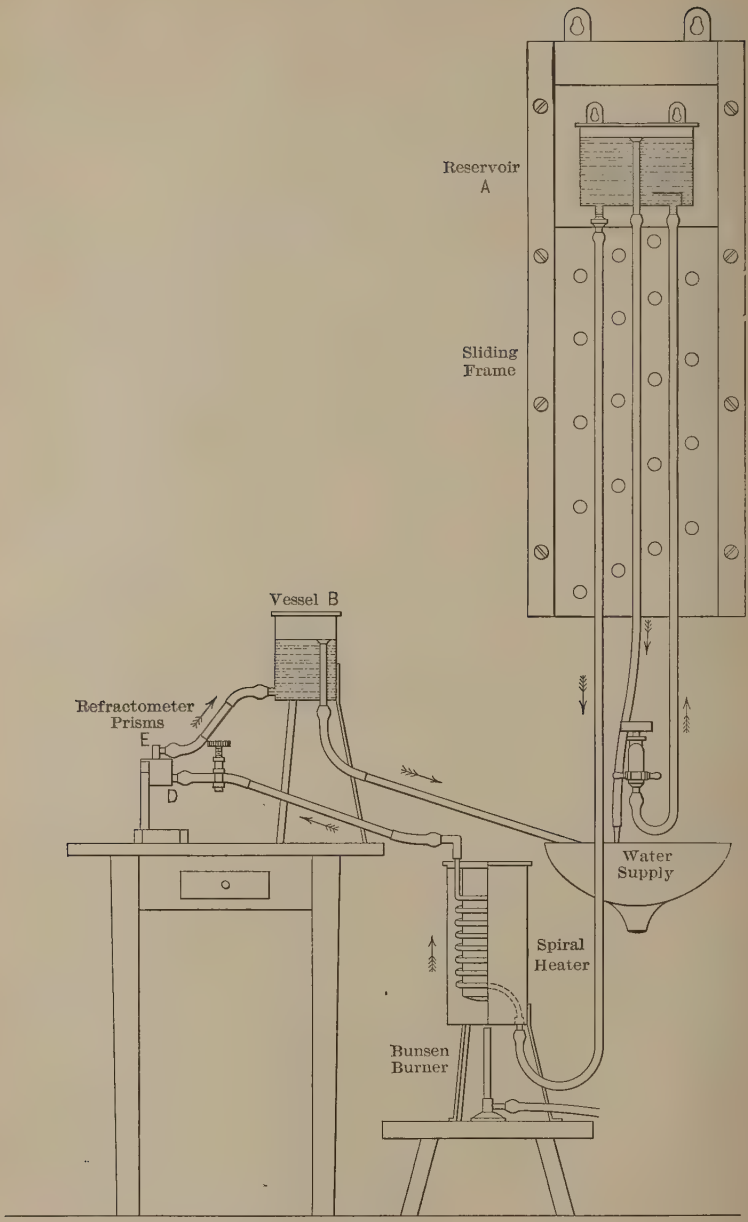



Fig. 34. — Zeiss spiral water-heater with pressure regulator.

attached to the upper prism by the capillary action of a drop of monobromonaphthalene; the polished end surface of the test plate is directed downwards to receive the reflected rays from the bright inner surface of the metal casing surrounding the lower prism. The average of several readings is taken, the prism being wiped clean and the plate reattached after each measurement. Care must be exercised not to confuse the reading in the reversed position of the sector scale. The average of the readings should not differ more than two points in the fourth decimal from the value marked upon the plate. Should greater differences than this occur, the refractometer should be adjusted. In some of the instruments the adjustment is made by moving the index of the sector scale with a setpin until it corresponds to the value marked upon the test plate. The border line of the field must remain meanwhile upon the intersection of the reticule, so that care must be exercised not to disturb the alidade while making the adjustment.

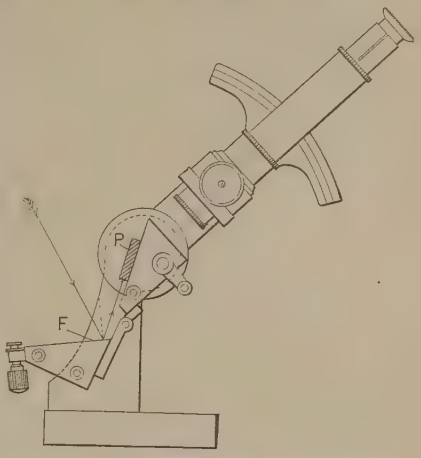



Fig. 35. — Verifying adjustment of refractometer by test plate.

In more recent forms of the Abbe refractometer the adjustment is made by moving the reticule instead of the index. The process is the reverse of that previously described. The alidade is first moved until the index of the scale corresponds to the reading of the test plate; then by means of a key the screw *K* (Fig. 31), which moves the reticule, is turned until the intersection of the cross threads coincides with the border line.

### Refractometer Tables for Sugar Solutions

A number of tables have been constructed which give the refractive indices of sugar solutions for different concentrations. The first of such tables was published in 1883 by Strohmer,* who showed also that a fixed relation existed between the refractive index and specific gravity

* Oest. Ung. Z. Zuckerind., **12,** 925; **13,** 185.

of sugar solutions. Using the method of least squares, Strohmer calculated this relation to be $n_D^{17.5°} = 1.00698 + 0.32717\,d$, in which $d$ is the specific gravity of the solution at 17.5° C.

In 1901 Stolle,* using a Pulfrich refractometer, constructed tables for sucrose, glucose, fructose, and lactose, a comparison of which showed that but very little variation existed in the refractive index of solutions of different sugars for the same concentration. The following table is made up from the observations of Stolle upon sucrose solutions of different concentrations.

TABLE XIV

*Giving Index of Refraction of Sugar Solutions*

| Concentration, grams to 100 c.c. | Specific gravity ($d$) $\frac{17.5°}{4°}$. | Per cent sucrose in solution. | Refractive index ($n$) 17.5°. | Refractive constant $\frac{n^2-1}{(n^2+2)\,d}$. |
|---|---|---|---|---|
| 0.9979 | 1.00241 | 1.00 | 1.33465 | 0.20612 |
| 4.0073 | 1.01406 | 3.95 | 1.33889 | 0.20615 |
| 12.0052 | 1.04484 | 11.49 | 1.35044 | 0.20617 |
| 17.9385 | 1.06736 | 16.81 | 1.35891 | 0.20621 |
| 25.0120 | 1.09420 | 22.87 | 1.36891 | 0.20617 |
| 35.0219 | 1.13194 | 30.94 | 1.38306 | 0.20610 |
| 45.8381 | 1.17246 | 39.10 | 1.39873 | 0.20619 |
| 55.0266 | 1.20651 | 45.61 | 1.41150 | 0.20602 |

The average value for the refractive constant (calculated by the formula of Lorenz and Lorentz) is 0.20614; from this it follows that the specific gravity ($d$) of sugar solutions may be calculated from the refractive index ($n$) by the equation $d = \frac{n^2 - 1}{(n^2 + 2) \times 0.20614}$.

In 1906 Tolman and Smith,† using an Abbe refractometer of latest construction, showed that "the refractometer is a satisfactory instrument for determining the soluble carbohydrates in solution under the same conditions as those under which specific gravity can be used, and in fact gives the same results; that it has many advantages over the specific gravity method in speed, ease of manipulation, and amount of sample required for the determination," and that the refractometer can be used for a great deal of work where quickness and approximate accuracy only are necessary. Tolman and Smith give the following table showing index of refraction at 20° C. and percentage of various carbohydrates in solution.

* Z. Ver. Deut. Zuckerind., **51**, 469.

† J. Am. Chem. Soc., **28**, 1476.

TABLE XV

*Giving Index of Refraction of Various Sugar Solutions of Different Concentration*

(Dried in vacuum at 70° C. to constant weight.)

| Index of refraction, 20° C. | Sucrose. | Maltose. | Commercial glucose. | Lactose. | Dextrin. |
|---|---|---|---|---|---|
| | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. |
| 1.3343 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1.3357 | 2.00 | 2.07 | 2.00 | 2.00 | 1.93 |
| 1.3402 | 5.00 | 5.07 | 5.00 | 5.13 | 4.87 |
| 1.3477 | 10.00 | 10.07 | 10.07 | 10.13 | 9.60 |
| 1.3555 | 15.00 | 15.12 | 15.06 | 15.13 | 14.13 |
| 1.3637 | 20.00 | 20.17 | 20.06 | ..... | 18.94 |
| 1.3722 | 25.00 | ..... | 25.00 | ..... | 23.71 |
| 1.3810 | 30.00 | ..... | 30.02 | ..... | 28.78 |
| 1.3902 | 35.00 | ..... | 35.03 | ..... | ..... |
| 1.3997 | 40.00 | ..... | 40.05 | ..... | ..... |
| 1.4096 | 45.00 | ..... | 45.04 | ..... | ..... |
| 1.4200 | 50.00 | ..... | 50.03 | ..... | ..... |
| 1.4306 | 55.00 | ..... | 55.02 | ..... | ..... |
| 1.4419 | 60.00 | ..... | 60.01 | ..... | ..... |
| 1.4534 | 65.00 | ..... | 65.01 | ..... | ..... |
| 1.4653 | 70.00 | ..... | 70.00 | ..... | ..... |
| 1.4776 | 75.00 | ..... | 75.00 | ..... | ..... |
| 1.4903 | 80.00 | ..... | 80.00 | ..... | ..... |
| 1.5034 | 85.00 | ..... | 85.00 | ..... | ..... |
| 1.5170 | 90.00 | ..... | 90.00 | ..... | ..... |

It will be seen from the above table that dextrin alone of the carbohydrates examined differs appreciably from sucrose in its index of refraction. Comparing the specific gravity $\frac{20°}{4°}$ of the above sucrose solutions with their refractive indices the method of least squares shows that $n_D^{20°} = 0.9509 + 0.3818\, d\frac{20°}{4°}$.

Tolman and Smith also studied the effects of temperature upon the refractive index of sugar solutions, and their results "show that the temperature correction for the specific gravity and the index of refraction are practically the same, and the table as given for Brix can be used for the index of refraction. The manner of using the table is the same. The reading of index of refraction is made at room temperature and this reading calculated to per cent of sugar, then the proper correction from the table calculated and applied."

Following the work of Tolman and Smith was that of Main* in 1907. Main was the first to demonstrate the practical utility of the Abbe refractometer in sugar-house work, and showed that the refractive index was an accurate measure of the moisture and total solids in all

* Int. Sugar Jour., 9, 481.

refinery products except the very lowest. The table of Main (Table 5, Appendix), which agrees almost exactly with that of Tolman and Smith, is the one employed by most sugar chemists at present. The indices give the percentage of water to 0.1 per cent from 100 per cent to 15 per cent; the percentage of water subtracted from 100 gives the corresponding percentage of total solids. Stanek* has prepared a table of temperature corrections for the table of Main, the figures of which show, as was indicated by Tolman and Smith, that the temperature corrections for specific gravity and refractive index are virtually the same (Table 6, Appendix).

Schönrock† of the Physikalisch-Technische Reichsanstalt in Berlin has made the most recent measurements of the refractive indices of sugar solutions. A preliminary report of Schönrock's determinations, which as regards attention to scientific detail are probably the most carefully conducted of any measurements thus far made, is given in Table XVI, in which $n$ is the refractive index at 20° C. for the two $D$ lines of sodium light (589.3 $\mu\mu$) and $w$ the water content of the solution.

TABLE XVI

*Giving Refractive Index and Water Content of Sugar Solutions*

| $n_D^{20°}$ | $w$ | $n_D^{20°}$ | $w$ | $n_D^{20°}$ | $w$ | $n_D^{20°}$ | $w$ |
|---|---|---|---|---|---|---|---|
| 1.3330 | 100 | 1.3639 | 80 | 1.3997 | 60 | 1.4418 | 40 |
| 1.3344 | 99 | 1.3655 | 79 | 1.4016 | 59 | 1.4441 | 39 |
| 1.3359 | 98 | 1.3672 | 78 | 1.4036 | 58 | 1.4464 | 38 |
| 1.3374 | 97 | 1.3689 | 77 | 1.4056 | 57 | 1.4486 | 37 |
| 1.3388 | 96 | 1.3706 | 76 | 1.4076 | 56 | 1.4509 | 36 |
| 1.3403 | 95 | 1.3723 | 75 | 1.4096 | 55 | 1.4532 | 35 |
| 1.3418 | 94 | 1.3740 | 74 | 1.4117 | 54 | 1.4555 | 34 |
| 1.3433 | 93 | 1.3758 | 73 | 1.4137 | 53 | ..... | ..... |
| 1.3448 | 92 | 1.3775 | 72 | 1.4158 | 52 | ..... | ..... |
| 1.3464 | 91 | 1.3793 | 71 | 1.4179 | 51 | ..... | ..... |
| 1.3479 | 90 | 1.3811 | 70 | 1.4200 | 50 | ..... | ..... |
| 1.3494 | 89 | 1.3829 | 69 | 1.4221 | 49 | ..... | ..... |
| 1.3510 | 88 | 1.3847 | 68 | 1.4242 | 48 | ..... | ..... |
| 1.3526 | 87 | 1.3865 | 67 | 1.4264 | 47 | ..... | ..... |
| 1.3541 | 86 | 1.3883 | 66 | 1.4285 | 46 | ..... | ..... |
| 1.3557 | 85 | 1.3902 | 65 | 1.4307 | 45 | ..... | ..... |
| 1.3573 | 84 | 1.3920 | 64 | 1.4329 | 44 | ..... | ..... |
| 1.3590 | 83 | 1.3939 | 63 | 1.4351 | 43 | ..... | ..... |
| 1.3606 | 82 | 1.3958 | 62 | 1.4373 | 42 | ..... | ..... |
| 1.3622 | 81 | 1.3978 | 61 | 1.4396 | 41 | ..... | ..... |

The above table shows no greater deviation at any reading than 4 in the fourth decimal place from the previous work of Main.

* Z. Zuckerind. Böhmen, **33**, 153.

† Z. Ver. Deut. Zuckerind., **61**, 421.

The use of the Abbe refractometer was extended to raw sugar cane products by Prinsen Geerligs and van West* who made a special study of the effect of impurities upon the refractive index of sugar solutions. Their results, in connection with observations upon low-grade Java molasses, show that the refractive index of impure sugar solutions is a much truer measure of the actual amount of dry substance present than the specific gravity. The refractometer table (Table 7, Appendix) of Geerligs† is established at 28° C. and is the one best adapted for tropical countries; the temperature corrections which accompany the table have a range from 20° to 35° C. When corrected to 20° C., Geerligs's results are identical with those of Tolman and Smith, and Main.

The use of the refractometer in the examination of sugar-beet products has been studied by Lippmann, Hübener, Lange, and many others. As in the case of sugar-cane products, the refractometer gives values for solid matter much closer to the true dry substance than specific gravity.

The percentage of moisture or dry matter in sugar products which have partly crystallized, such as massecuites, moist sugars, etc., can be made upon the refractometer after dissolving all soluble matter with a known amount of water.

*Example.* — 10 gms. of massecuite were dissolved in 10 c.c. of hot distilled water, the weight of mixture after cooling to 20° C. being brought to 20 gms. by addition of distilled water of 20° C. The refractive index of the mixture was 1.4107, which according to Main's table indicates 54.45 per cent water. 54.45 per cent of 20 gms. = 10.89 gms. water in mixture. 10.89 − 10 (gms. water added) = 0.89 gm. water in original massecuite, or 8.90 per cent.

Hardin has made comparative determinations of the moisture in different grades of sugar by drying and by the refractometer with the following results:

| Grade of sugar. | Refractive index, 20° C. (1 part sugar +1 part distilled water). | Per cent of water. | |
|---|---|---|---|
| | | By refractometer. | By drying to constant weight. |
| | | Per cent. | Per cent. |
| Refined sugar | 1.4200 | 0.10 | 0.05 |
| Hawaiian centrifugal | 1.4199 | 0.20 | 0.45 |
| Philippine mats (dried out) | 1.4197 | 0.40 | 0.82 |
| Java centrifugal | 1.4190 | 1.00 | 0.82 |
| Louisiana centrifugal | 1.4189 | 1.10 | 1.05 |
| Cuban centrifugal | 1.4181 | 1.90 | 1.93 |
| Muscovado | 1.4179 | 2.10 | 2.40 |
| Molasses sugar | 1.4172 | 2.70 | 2.83 |
| Molasses sugar | 1.4139 | 5.90 | 5.54 |

* Archief Java Suikerind. (1907), **15**, 487. † Int. Sugar Jour., **10**, 69–70.

The variations in the results by the two methods are in both directions, and may have been due either to the presence of trash in the sugar or to the influence of non-sugars. Since the refractometer only indicates the percentage of dissolved solids, any insoluble matter which is present in the weighed sample will introduce an error in the calculation.

Insoluble suspended matter in sugar solutions, if present in large amounts, will darken the field of the refractometer and interfere with the adjustment of the border line. In such cases the solution must be filtered.

**Examination of Dark-colored Sugar Solutions with the Refractometer.** — In the examination of dark-colored sugar solutions, molasses, sirups, extracts, etc., by means of the refractometer, it is not always possible for the compensator to eliminate completely the effects of dispersion; the border line of the field is then more or less blurred and a sharp adjustment to the intersection of the reticule becomes a matter of some difficulty. In solutions which are not too strongly colored this trouble may be remedied by bringing the border line to the point of intersection alternately from each side of the field; the average of the readings thus obtained will correct to a large extent the errors of faulty adjustment. Some authorities have recommended with dark solutions to adjust the compensator to a colored border, selecting the color most sensitive to the observer's eye; this, however, is not very satisfactory, and if the blurring of the border line is excessive, the color of the solution must be reduced by some method of dilution or clarification.

In the dilution of impure sugar products with water an error will be introduced in the refractometer reading in the same manner as in the determination of specific gravity, owing to the difference in contraction between solutions of sugar and of the accompanying impurities (page 35).

A study of the errors resulting from unequal contraction, when dilution is employed in densimetric and refractometric methods of analysis, has been made by Stanek.* Fifty per cent solutions of betaine and of various organic salts of sodium and potassium were prepared. These solutions were then diluted with known weights of water and the per cent of dry substance determined from the degrees Brix, from the refractive indices according to Main's table, and by drying on sand in a Soxhlet oven at 102° C. A few of the results are given in the following table:

* Z. Zuckerind. Böhmen, **34**, 5.

TABLE XVII

*Comparative Determinations of Solids by Brix, Refractometer and Drying at* 102°

| Substance taken. | True dry substance. | Dry substance by | | |
|---|---|---|---|---|
| | | Degrees Brix. | Refractometer. | Drying at 102°. |
| | Per cent. | Per cent. | Per cent. | Per cent. |
| Betaine (anhydrous) | 5 | 2.2 | 5.10 | 5.05 |
| | 10 | 4.3 | 10.20 | 10.01 |
| | 25 | 10.8 | 24.15 | 25.03 |
| Sodium formate | 5 | 8.1 | 4.60 | 4.99 |
| | 10 | 15.6 | 8.85 | 10.04 |
| | 25 | 37.7 | 20.35 | 25.05 |
| Potassium formate | 5 | 7.3 | 3.60 | 5.00 |
| | 10 | 14.28 | 7.20 | 9.97 |
| | 25 | 35.7 | 17.20 | 25.09 |
| Sodium acetate | 5 | 6.7 | 5.00 | 4.97 |
| | 10 | 13.1 | 9.70 | 9.99 |
| | 25 | 31.1 | 22.70 | 25.00 |
| Potassium acetate | 5 | 6.6 | 5.00 | 5.00 |
| | 10 | 12.8 | 8.25 | 10.07 |
| | 25 | 30.4 | 19.75 | 25.15 |
| Sodium butyrate | 5 | 4.75 | 4.90 | 4.90 |
| | 10 | 9.4 | 10.25 | 9.89 |
| | 25 | 22.9 | 24.35 | 24.94 |
| Sodium lactate | 5 | 6.3 | 5.00 | 5.10 |
| | 10 | 12.3 | 10.00 | 10.07 |
| | 25 | 30.2 | 24.05 | 25.05 |
| Potassium lactate | 5 | 6.3 | 4.85 | 5.18 |
| | 10 | 12.5 | 9.10 | 10.13 |
| | 25 | 30.3 | 21.65 | 25.20 |
| Sodium glutaminate | 5 | 6.8 | 6.40 | 5.05 |
| | 10 | 13.2 | 12.50 | 10.23 |
| | 25 | 31.1 | 30.05 | 26.41 |
| Potassium glutaminate | 5 | 6.7 | 5.90 | 5.03 |
| | 10 | 13.1 | 11.50 | 10.24 |
| | 25 | 30.65 | 27.70 | 25.27 |

It will be noted from the above that the refractometer gives a much closer approximation to the true dry substance than the degrees Brix, the refractometer yielding usually lower results and the degrees Brix higher. It is also seen that the sodium salts of organic acids give higher results by both methods than potassium salts. Contraction upon dilution is noted in every case, the results corrected for dilution

being higher according to the amount of water added. The usual effect of this contraction is to make the error in estimating non-sugars less by the refractometer and greater by degrees Brix. Neither of these methods for estimating non-sugars approaches in point of accuracy the method of actual drying.

The errors in determining the refractive index of dark impure sugar solutions, resulting from dilution with water, may be largely eliminated by employing the method of Tischtschenko,* which consists in reducing the color of the product by means of a solution of pure sucrose of about the same density as the liquid to be examined. The disturbing influences of color dispersion in the refractometer field are in this way overcome without the errors of contraction. The method of operation is as follows: A known weight ($a$) of the molasses, sirup, etc., is intimately mixed with a known weight ($b$) of pure sugar solution, whose sugar content ($p$) has been previously determined by means of the refractometer. The refractive index of the mixed solution is then determined and the corresponding percentage ($P$) of dry substance found from the table. The percentage of dry substance ($x$) in the molasses, sirup, etc., is then calculated by the formula $ax + bp = (a + b)P$,

whence $$x = \frac{(a + b)P - bp}{a}.$$

*Example.* — Weight of beet molasses ($a$) = 14.1028 gms.
Weight of sugar sirup ($b$) = 13.2438 gms.
Sugar in sirup ($p$) = 51.3 per cent.
$n_D^{20°}$ of mixture = 1.4538 = 34.87 per cent water (Main's table).
Solids of mixture ($P$) = 100 − 34.87 = 65.13 per cent.

Substituting these values in the formula, $x = 78.12$ per cent solids in molasses. The method by water dilution gave 79.11 per cent. Direct determination by drying gave 77.80 per cent.

If a sugar sirup of greater density had been used for mixing, the value of $x$ would have been more close to the result by direct determination.

If equal weights of molasses and sugar solution are used in Tischtschenko's method, then $a = b$ in the formula, whence $x = 2P - p$; the labor of calculation is thus considerably reduced. In using the method, the mixture of molasses and sugar solution must be perfectly homogeneous. Care must also be exercised, as in all cases, that no air bubbles are inclosed with the liquid between the prisms. A com-

* Z. Ver. Deut. Zuckerind., **59**, 103.

parison of results in determining dry substance in different samples of beet molasses by various methods is given by Lippmann* in the following table:

TABLE XVIII

*Comparative Determinations of Solids in Beet Molasses by Drying, Specific Gravity, and Refractometer*

| Number. | By direct determination. | By degrees Brix. | By refractometer. | |
|---|---|---|---|---|
| | | | Water dilution. | Tischtschenko's method. |
| 1 | 76.78 | 78.90 | 77.90 | 76.80 |
| 2 | 77.95 | 79.80 | 78.50 | 78.00 |
| 3 | 76.22 | 78.60 | 77.00 | 76.10 |
| 4 | 77.85 | 79.30 | 78.60 | 77.90 |
| 5 | 77.05 | 79.40 | 78.20 | 77.30 |
| 6 | 77.55 | 79.20 | 78.10 | 77.80 |
| 7 | 77.97 | 79.90 | 78.60 | 78.30 |
| 8 | 77.32 | 79.30 | 78.20 | 77.70 |
| 9 | 77.50 | 79.30 | 78.60 | 77.88 |
| 10 | 77.31 | 79.60 | 78.40 | 77.70 |
| 11 | 76.58 | 78.90 | 77.70 | 77.00 |
| 12 | 76.94 | 79.20 | 77.90 | 77.40 |
| 13 | 77.43 | 79.60 | 78.50 | 77.90 |
| 14 | 76.53 | 78.90 | 77.70 | 77.00 |
| 15 | 77.82 | 80.00 | 79.00 | 78.30 |
| 16 | 77.90 | 80.20 | 78.90 | 77.40 |
| Average | 77.29 | 79.38 | 78.24 | 77.53 |

It will be noted from the above that the average error of estimating dry substance in the 16 samples of beet molasses was, by degrees Brix, +2.09 per cent; by refractometer, using water dilution, +0.95 per cent; and by refractometer, using Tischtschenko's method, only +0.24 per cent.

Another method of correcting the disturbances in refractometer work due to color of solution is by clarification. Lead subacetate is the reagent most generally employed for this purpose. The use of this and similar salts must be limited, however, to the greatest possible minimum, since the excess of salt remaining in the clarified solution causes an increase in the refractive index. In the following experiments made by Rosenkranz† at the Berlin Institute for Sugar Industry, the effect of increasing the quantity of subacetate is shown upon the refractive index of a molasses containing 78.59 per cent dry substance and diluted 1:1, inclusive of the lead solution added.

* Deut. Zuckerind., **34,** 402.

† Z. Ver. Deut. Zuckerind., **58, 195.**

| Lead subacetate. | Specific gravity, dilute solution, 20°. | Calculated Brix of original molasses. | Refractive index, dilute solution. | Dry substance, dilute solution (Main's table). | Calculated dry substance, original molasses. |
|---|---|---|---|---|---|
| c.c. | | | | | |
| ..... | 1.1813 | 81.9 | 1.3994 | 39.85 | 79.70 |
| 5 | 1.1865 | 84.0 | 1.4003 | 40.3 | 80.60 |
| 10 | 1.1912 | 85.7 | 1.4009 | 40.6 | 81.2 |
| 12.5 | 1.1951 | 87.2 | 1.4022 | 41.3 | 82.6 |

Another material recommended by Lippmann for decolorizing dark sirups, etc., for the refractometer is "Decrolin," the zinc salt of formaldehyde sulphoxylic acid, $CH_2OH.O.SO.Zn.OH$. One to two per cent of Decrolin is used and the liquid heated to about 55° C. to hasten solution and decolorization.

For the refractometric examination of turbid beet juices, etc., Herzfeld* has recommended the addition of a few drops of 10 per cent acetic acid, heating for 2 minutes at 80° C. to coagulate albuminoids, and filtering. With beet juices the effect of dilution (1 to 5 per cent) is compensated by the greater refractive index of the 10 per cent acetic acid used, as shown in the following experiment:

| 10 c.c. beet juice. | Refractive index, $n_D^{20°}$. | Dry substance by Main's table. |
|---|---|---|
| +0.5 c.c. water..... | 1.3583 | 16.75 |
| +0.5 c.c. acetic acid (10 per cent).... | 1.3595 | 17.45 |
| +0.25 c.c. water..... | 1.3588 | 16.95 |
| +0.25 c.c. acetic acid (10 per cent).... | 1.3591 | 17.20 |
| +0.10 c.c. water..... | 1.35905 | 17.15 |
| +0.10 c.c. acetic acid (10 per cent).... | 1.35905 | 17.15 |

### The Immersion Refractometer

A second form of instrument which is used for determining the refractive power of sugar solutions is the immersion refractometer, the Zeiss model of which is shown in Fig. 36. While this instrument has a narrower range than the Abbe apparatus, the scale being adapted only for solutions containing from 0 to 21.7 per cent sugar, it gives a much sharper border line, thus allowing a greater magnification in the telescope, with a corresponding increase in the accuracy of observation. In the immersion refractometer there is no sector; the scale is placed below the eyepiece of the telescope, the latter, unlike the Abbe refractometer, being rigidly connected with the prism.

* Z. Ver. Deut. Zuckerind., **58**, 197.

The principle of the immersion refractometer is the same as that of the Abbe instrument, being based upon an observation of the border line of total reflection. In Fig. 37, $G$ is a cylindrical glass prism with its refracting surface $DE$ immersed in the liquid $W$ contained in the glass beaker $V$. If we suppose light to pass through the top of the prism from the surface $AB$, the parallel rays $sP$, $s'P'$, $s''P''$, etc., will

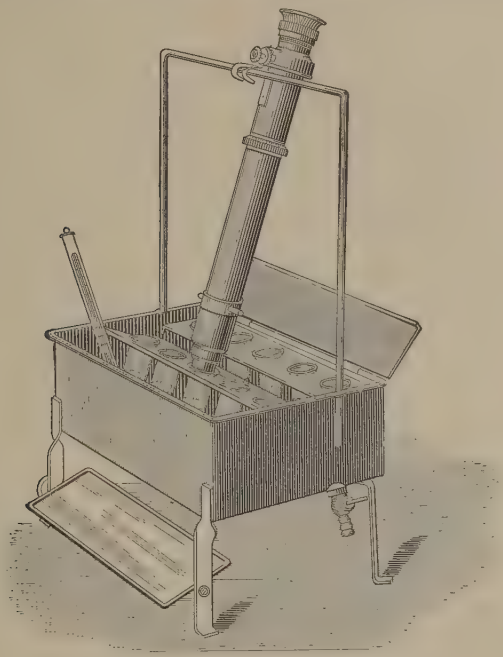


Fig. 36. — Zeiss immersion refractometer.

be refracted in the liquid in the direction $PM$, $P'M'$, $P''M''$, etc. By increasing the angle of incidence for the parallel rays upon the surface $DE$, a point is reached where the parallel rays $rP$, $r'P'$, $r''P''$, etc., are refracted along the surface of the prism towards $D$. This is the border line of total reflection as explained under Fig. 30, where the angle of refraction is 90°. In the use of the immersion refractometer the course of the light is in the reversed direction to that just described, being reflected from the mirror $HK$ through the bottom of the beaker $V$ so as to pass as nearly parallel as possible to the oblique surface of the prism. The rays of light which coincide with the surface $DE$ form

the border line for total reflection and are refracted upward through the prism as the parallel rays $Pr$, $P'r'$, $P''r''$, etc., which, being condensed by the objective $O$ of the refractometer telescope upon the point $x$ of the scale $S$, form the border line for observation; the rays of light which may strike the prism surface obliquely, as $MP$, $M'P'$, $M''P''$, etc., are refracted in the direction $Ps$, $P's'$, $P''s''$, etc., and being condensed by the objective between $x$ and $y$ cause this part of the scale to be illuminated. There being no possible angle of refraction for light in the prism greater than that for the border line of total reflection, the part of the scale between $x$ and $z$ remains in shadow.

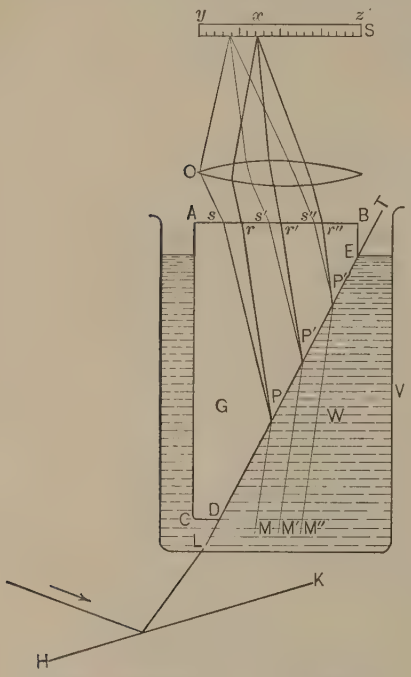



Fig. 37. — Illustrating principle of immersion refractometer.

As in the Abbe refractometer, the border line on account of differences in dispersion is fringed with color and must be corrected by a compensator in the manner described on p. 57. The compensator is placed at $A$ (Fig. 38) between the objective $O$ and the prism $P$ and is rotated by the milled ring $R$ until the border line upon the scale becomes sharp and colorless. The position of the border line upon the scale marks the reading for the whole division; the fractional division is determined by rotating the micrometer screw $Z$, which controls the scale, until the whole division previously noted is brought into contact with the border line. The reading of the micrometer drum shows the fractional division which remains to be added. Readings can be made by careful observers to agree within 0.1 scale division, which corresponds to 3.7 of the fifth decimal of the refractive index. This exceeds considerably in accuracy the reading of the Abbe refractometer.

The adjustment of the Zeiss immersion refractometer scale is made by means of distilled water, which should give a reading of 15 at

17.5° C. The adjustment, however, can be made at other temperatures according to the following table.

The correctly adjusted refractometer should show for distilled water:

| At a temperature of ..... | 10° C. | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 17.5 | 18 | 19° C. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| The scale division..... | 16.3 | 16.15 | 16.0 | 15.85 | 15.7 | 15.5 | 15.3 | 15.1 | 15.0 | 14.9 | 14.7 |

| At a temperature of ..... | 20° C. | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30° C. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| The scale division..... | 14.5 | 14.25 | 14.0 | 13.75 | 13.5 | 13.25 | 13.0 | 12.7 | 12.4 | 12.1 | 11.8 |

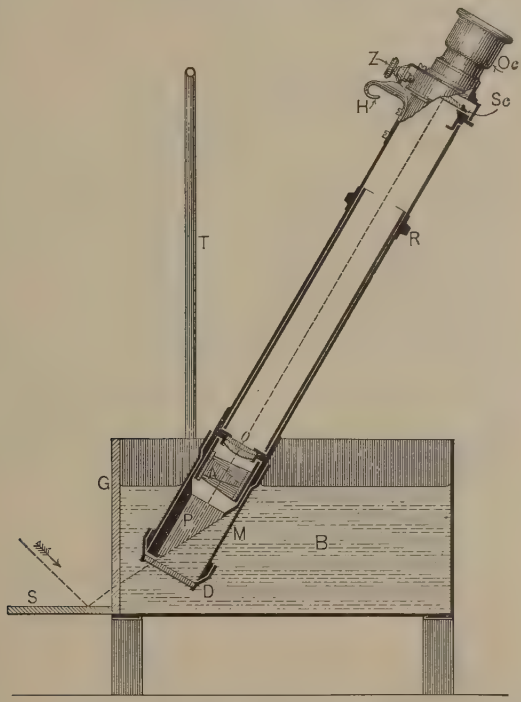



Fig. 38. — Showing inner construction of immersion refractometer.

Should the average of several careful readings differ by more than 0.1 division from the reading in the above table for the temperature of testing, the scale should be readjusted. This is done by first setting the micrometer at 10; then by inserting a setpin in the hole of an

adjusting screw inside the micrometer drum and turning anticlockwise, the border line of the field is made to agree with the whole scale division corresponding to the temperature of the water. The loosened micrometer drum is now turned until its index marks the proper decimal; holding it firmly in this position, the nut which governs the micrometer is retightened. The new adjustment should be controlled by repeated readings.

The readings of the Zeiss immersion scale extend from −5 to +105, and are converted into refractive indices or into percentages of sugar

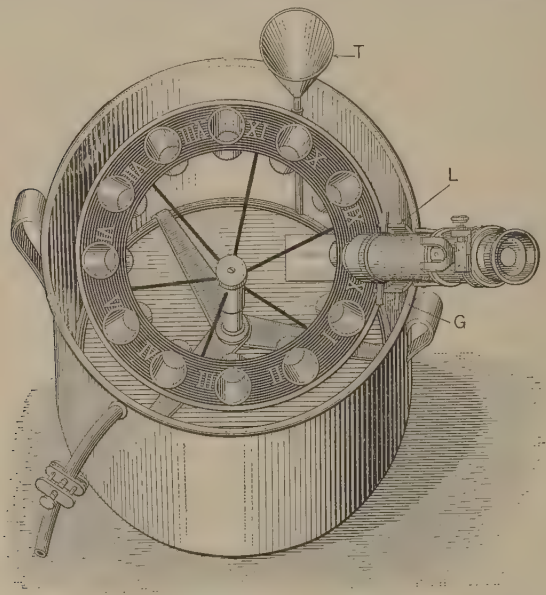



Fig. 39. — Tempering bath for immersion refractometer.

by means of special conversion tables which accompany the instrument. Sugar tables for the immersion refractometer have been prepared by Hübener;* these give the sucrose values of the scale from 15 to 106 with percentages of sucrose from 0.00 to 21.71. Each 0.1 division of the scale corresponds to about 0.02 per cent sucrose or other sugar, and readings can be made with this degree of exactness. (See Table 8, Appendix.)

For controlling the temperature of the water bath, containing the

* Deut. Zuckerind., **33**, 108.

beakers of solution for the immersion refractometer, the spiral heater and water-pressure regulator previously described may be used. A tempering bath holding 10 liters of water and with a revolving frame for 12 beakers (shown in Fig. 39) is also recommended. When the proper temperature has been reached in the beakers the solutions are read in sequence, the refractometer prism being wiped dry after each immersion. When large numbers of solutions are to be tested, each solution as soon as read is replaced by a beaker of fresh solution, thus giving sufficient time for regulation of temperature without interruption of work.

When only a few cubic centimeters of solution are available or when the liquids to be examined consist of dark-colored sirups, molasses, extracts, etc., the immersion prism is fitted with an auxiliary prism held in position by means of a metal beaker and cover. The method of use is somewhat similar to that of the Abbe refractometer; the hypotenuse surface of the auxiliary prism is covered with a few drops of solution and then inserted in the beaker against the face of the immersion prism so that a thin layer of liquid is spread between the two.

The remarks upon illumination under the Abbe refractometer also apply to the immersion instrument.

As to the particular choice of refractometer for the sugar laboratory, the chemist must be guided by his requirements. The Abbe refractometer has the widest range, is adapted to smaller quantities of solution, and is more convenient to operate. The immersion refractometer, however, is more accurate in adjustment and much less expensive. For general work the Abbe instrument will be found more useful; for more limited operations upon solutions below 20° Brix, such as beet and cane juices, sweet waters, etc., the immersion instrument possesses certain advantages.

# CHAPTER V

## POLARIZED LIGHT, THEORY AND DESCRIPTION OF POLARIMETERS

In order to arrive at a sufficiently clear understanding of the optical principles which underlie the construction and manipulation of polariscopes, a brief reference must be made to the physical theories of light.

According to the undulatory theory of Huyghens, light consists of vibrations or wave motions of the luminiferous ether, the imponderable medium which pervades all space and penetrates all matter.

Waves of light, contrary to those of sound, vibrate transversally instead of longitudinally. In Fig. 40 a graphic representation is given

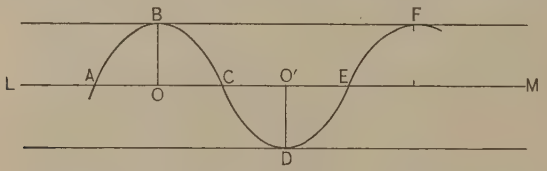



Fig. 40. — Illustrating principle of a light wave.

of a light wave vibrating transversally to the direction of motion $LM$. The plane of vibration of ordinary light takes all possible positions about this line of motion. The distance $OB$ or $O'D$ from the middle to the extremity of an oscillation is known as the amplitude of the wave. The distance from $A$ to $E$ (points in the same phase) is known as the wave length ($\lambda$), which for light is expressed in millionths of a millimeter ($\mu\mu$). The number of waves per second is called the rate of vibration ($N$). If the velocity of light through a homogeneous medium be $V$, then $N = \frac{V}{\lambda}$.

According to Maxwell's electromagnetic theory, which has since been confirmed by the work of Hertz, there are two sets of transverse vibrations in the transmission of a ray of light, the one an electric displacement of the ether, and the other a magnetic displacement, the planes of these being perpendicular to each other.

The intensity of a ray of light is proportional to the square of the

amplitude; the color depends upon the rate of vibration of the ether wave. The color of light may, therefore, be expressed mathematically in terms of the rate of vibration $N$ or of its wave length $\lambda$. The values of $N$ and $\lambda$ for the average ray in each color of the spectrum are given in the following table:

TABLE XIX.

| Color. | Rate of vibration per second ($N$). | Wave length ($\lambda$) in millionths of a millimeter ($\mu\mu$). |
|---|---|---|
| | Billions. | |
| Red | 437 | 683 |
| Orange | 485 | 615 |
| Yellow | 534 | 559 |
| Green | 582 | 512 |
| Blue | 631 | 473 |
| Indigo | 679 | 439 |
| Violet | 728 | 410 |

The human eye is sensitive to light of vibration periods between about 366 and 804 billion per second, and of wave lengths between about 820 $\mu\mu$ and 373 $\mu\mu$. Ether waves of greater length than 820 $\mu\mu$ constitute the so-called infra-red or heat rays, and those of shorter length than 373 $\mu\mu$ the so-called ultra-violet or chemical rays.

Light of definite wave length is exceedingly important in making polariscopic measurements, and this is secured by using incandescent salts of certain metals, as sodium or lithium, which give bright spectral lines whose wave lengths are absolutely defined. The prominent lines of the different elements are usually designated by the letters of the alphabet, which have been adopted to mark their positions in the solar spectrum. For the sodium line* $D$, to which nearly all polariscopic measurements are referred, $\lambda = 589.3$ $\mu\mu$.

The vibrations of ordinary light proceed in an infinite number of planes. By means of various special contrivances it is possible, however, to affect a beam of light so that the electric and magnetic vibrations will each proceed in a single plane. Such light is said to be plane-polarized; the plane to which the electric vibration of the waves is perpendicular is called the plane of polarization.

The polarization of light was first noticed by Huygens in 1678, while studying the refraction of light in a crystal of Iceland spar. No satisfactory explanation of the phenomenon was made, however, until

* The sodium line is double; the component $D_1$ has a wave length of 589.6 $\mu\mu$ and the brighter component $D_2$ a wave length of 589.0 $\mu\mu$. The average wave length of the two lines, 589.3 $\mu\mu$ (more exactly 589.25 $\mu\mu$), is the value taken for $D$.

Malus, in 1808, discovered that the polarization noticed by Huygens in Iceland spar could also be produced by reflection.

**Polarization by Reflection.** — If a beam of light (as *LO* in Fig. 28) fall upon the smooth surface of a transparent substance, it is decomposed into reflected and refracted rays. The reflected rays at a definite angle of incidence are completely polarized, the plane of the lines of incidence and reflection being the plane of polarization.* These observations, according to Fresnel and Arago, could be explained only by supposing that the vibrations in a light wave are tranverse to the direction of motion, and that during reflection these vibrations are reduced to a single plane, which is perpendicular to the plane of polarization.

The angle of incidence at which reflected light is completely polarized is called the polarizing angle, and varies according to the refractive power of the reflecting substance. This relationship is expressed by Brewster's law, viz.: The tangent of the polarizing angle is equal to the index of refraction for the reflecting substance, or $\tan i = n$. The polarizing angle of glass ($n = 1.54$) is accordingly about 57 degrees.

**The Nörrenberg Apparatus.** — A simple apparatus for producing and studying polarized light is that of Nörrenberg, shown in Fig. 41. *A* and *B* are two mirrors of black glass; the upper mirror *B* can be rotated by the crank *D* around the vertical axis of the instrument, the angular displacement being indicated upon a divided circle *S*. The planes of the two mirrors are first placed parallel, at an angle of 45 degrees to the vertical, and a beam of light is allowed to fall upon the mirror *A* at an angle of incidence of 57 degrees. The reflected beam is then completely polarized and, passing upward, is reflected from mirror *B* upon the screen *C*, where it appears as a bright spot. With the mirrors parallel, the planes of incidence and reflection, and hence of polarization, coincide for each surface. Without changing its inclination, the mirror *B* with its screen *C* is rotated by the crank *D* around the vertical axis. The plane of incidence and reflection for the beams of polarized light at mirror *B* no longer coincide with that at mirror *A*; the intensity of the spot of light upon the screen accordingly begins to diminish until, after a revolution of 90 degrees, the screen is perfectly dark, all the light being refracted and absorbed in the mirror *B*. In the latter position the planes of incidence, and hence of polarization, for the light of the two mirrors are at right angles, and the mirrors are said to be crossed. By turning *D* in the same direction the spot of light

* The refracted rays of light are also polarized, but not completely; most of the refracted rays, however, are polarized in one direction, their plane of polarization being perpendicular to that of the reflected rays.

reappears upon the screen, and after 180 degrees again reaches maximum brilliancy, in which position the planes of incidence and of polarization again coincide in both mirrors; at 270 degrees, when these planes are again at right angles, the spot of light is reëxtinguished.

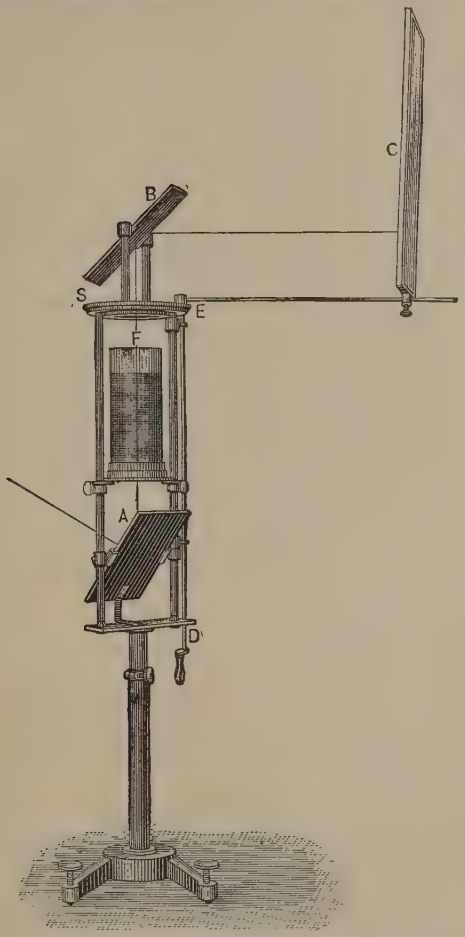



Fig. 41. — Nörrenberg's polarizing apparatus.

If at one of the points of extinguishment of light upon the screen the glass cylinder *F* containing a solution of sucrose or other optical active sugar be inserted in the path of the light rays reflected from *A*,

the illumination upon the screen will reappear. The plane of polarization of the light reflected from *A* must, therefore, have been rotated by the sugar solution through a certain angle in order that reflection could take place from *B*; by turning *D* until the plane of polarization for the light upon *B* is again brought perpendicular to the plane of incidence, the point of maximum darkness is reëstablished. By measuring upon *S* the positions of maximum darkness, before and after inserting the cylinder, the angle through which the sugar solution has rotated the plane of polarized light can be measured. In the Nörrenberg apparatus the mirror *A* for polarizing the light is called the polarizer and the mirror *B* for measuring rotation the analyzer.

**Polarization by Double Refraction.** — Of the several contrivances available for producing plane polarized light, a modified crystal of Iceland or calc spar is the only one used in the construction of polariscopes and saccharimeters. Calc spar is a clear, transparent mineral which cleaves readily into rhombohedra. If a small object be viewed through such a rhombohedron, the image will be doubled. Rays of light in passing through the crystal undergo "double refraction." The phenomenon is noticeable in any position of the calc-spar rhombohedron except in a direction parallel to the diagonal joining the two opposite

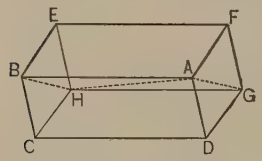



Fig. 42. — Calc spar rhombohedron.

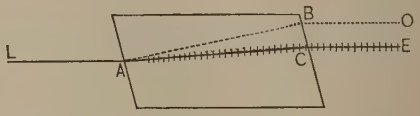



Fig. 43. — Illustrating double refraction of light in calc spar.

obtuse corners, known as the optical axis. Any plane including the optical axis and perpendicular to the face of the crystal is called an axial plane or principal section.

In the rhombohedron of calc spar, in Fig. 42, the direction *AH* is the optical axis. The plane *ABHG* (or any parallel plane) perpendicular to the face *AFGD* is an axial plane or principal section to that face.

If a beam of light *LA* fall upon the surface of such a rhombohedron (Fig. 43), it is resolved into two rays, the ordinary ray *ABO* and the extraordinary ray *ACE*. Both of these rays emerging from the crystal are polarized, their planes of polarization being perpendicular to each other.

**The Nicol Prism.** — Before a crystal of calc spar can be utilized for polariscope construction it must be modified so as to eliminate one set of the component rays. The best known method (that of Nicol) is the following: A rhombohedron *ABCD* (Fig. 44) is selected whose length is about three times the width. At each end of the crystal, wedge-shaped sections *BFC* and *ADE* are removed so as to reduce the acute angles *DAB* and *BCD* of the axial plane from 71 degrees to 68 degrees. The crystal is then divided by the plane *FGEH* perpendicular to the two modified end faces. The cut surfaces are then polished and reunited with Canada balsam.* The sides of the prism thus obtained are afterwards blackened and the whole is mounted by means of cork and wax in a metal tube.

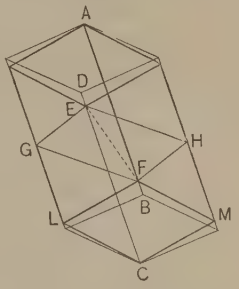



Fig. 44. — Illustrating construction of Nicol prism.

Let *AFCE* represent a principal section of the Nicol prism (Fig. 45). A beam of light *LT* entering parallel to the long sides of the prism is resolved into two component rays; the component most refracted (the ordinary ray) meets the film of balsam *EF* at such an angle that it is completely reflected to the side of the prism, where it is absorbed by the dark coating. The other component (the extraordinary ray), whose vibrations are in the plane of the principal section, is less refracted and, passing through the film of balsam, emerges in a polarized condition

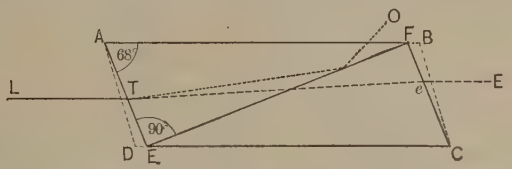



Fig. 45. — Illustrating polarization of light by a Nicol prism.

from the end surface of the Nicol at the point *e*. With respect to the end surface of the Nicol *FCLM* (Fig. 44), the electric vibrations of the emergent light are in the plane of the principal section, i.e., in the direction of the short diagonal *FC*; the plane of polarization is in the direction of the long diagonal *LM*.

* "Iceland spar is rather friable, and in practice it is found easier to grind away half of the rhomb instead of cutting it, as generally described. The remaining halves of two rhombs thus ground are then cemented together." — Preston, "Theory of Light," third edition, p. 319.

In the discussion of polarized light, it makes no difference which plane is taken for reference, provided it be always the same. In future pages the terms vibrate, vibration, plane of vibration, etc., refer entirely to the electric displacements in the transmission of light. With this understanding, the statement of Fresnel, which is followed in nearly all works upon polarimetry, — that the plane of vibration of light is perpendicular to the plane of polarization, — can be retained without confusion.

**The Glan Prism.** — The type of Nicol prism which is the most scientifically perfect and the one most used at present in constructing polariscopes and saccharimeters is that of Glan. In constructing this prism the opposite obtuse corners of a calc-spar rhombohedron (as *ABCDEF*, Fig. 46) are cut off by planes *PQR* and *STF* perpendicular to the optical axis which passes through the point *X*. From this section a rectangular prism *LMNO* is sawed out, which is then cut in half along a plane through *MN*. After polishing, the cut halves are cemented together again by Canada balsam and mounted as in an ordinary Nicol. The great advantages of the Glan prism over the ordinary Nicol are that the rays of light enter the prism perpendicular to the end surface and at right angles to the optical axis, thus securing the greatest amount of light capacity per unit of length.

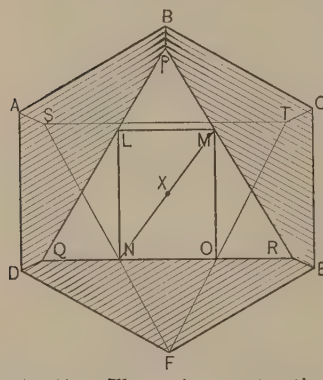


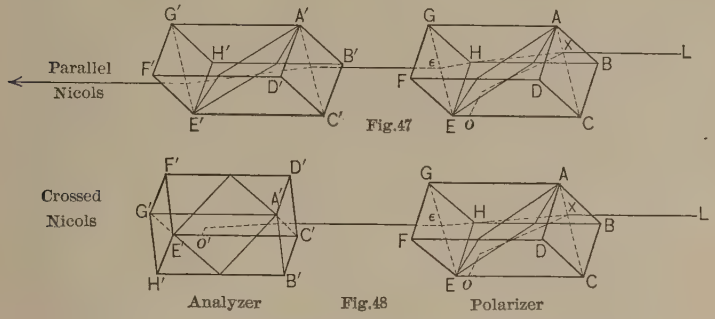
Fig. 46. — Illustrating construction of a Glan prism.

## Principle and Construction of Polarimeters

**Polarizer and Analyzer.** — A combination of two Nicol prisms, called the polarizer and analyzer, constitutes the essential feature of every polariscope. The function which these two parts play can best be understood from the following diagram (Figs. 47 and 48).

The polarizer, which is stationary, is represented by the prism *ABCDEFGH*, whose axial plane lies through *ACEG*. A beam of light entering from *L* at the point *x* is doubly refracted; the ordinary rays are eliminated at *o*, while the extraordinary rays emerge at *e*, vibrating in the axial planes of the prism, with the plane of polarization parallel with the plane *BDFH*. If the emergent polarized light now enter a second prism *A'B'C'D'E'F'G'H'* (the analyzer), which can be rotated

about its long axis, its course will remain unimpeded only so long as it can continue to vibrate in the same axial plane. If the analyzer be rotated about its long axis, the light which enters from the polarizer is doubly refracted and only that component which vibrates in the




Figs. 47 and 48. — Illustrating principle of polarizer and analyzer.

plane of the principal section emerges. As the analyzer is rotated the intensity of the emergent light diminishes until after a quarter revolution it is completely extinguished; in this position the axial planes of polarizer and analyzer are perpendicular to one another and the two prisms are said to be crossed (Fig. 48). If the rotation of the analyzer be continued, light will again begin to emerge, until after a half-revolution, when the axial planes are again parallel, the original intensity will be restored.

The amount of light which will pass through the analyzer for any position of its axial plane with reference to the polarizer may be readily calculated by referring to Fig. 49.

Let $AB$ be the axial plane of the polarizer (always stationary) and $CD$ any given position of the axial plane of the analyzer, the two planes forming the angle $DOB$. From $O$ lay off any distance $OP$ as the amplitude of the light emerging from the polarizer. From $P$ erect $PL$ perpendicular to $CD$; then the line $OL$ represents the amplitude of the light emerging from the analyzer and $PL$ the amplitude of the light extinguished in the analyzer. As regards the relation in intensity, this is proportional to the squares of the amplitudes: $\overline{OP}^2 = \overline{OL}^2 + \overline{PL}^2$.

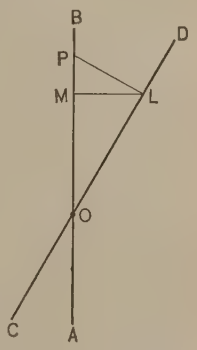



Fig. 49. — Showing proportion of light extinguished by analyzer.

If we erect $LM$ perpendicular to $AB$ and call the intensity of the light emerging from the polarizer $OP$, then the intensity of the light emerging from the analyzer will be represented by $OM$ and the intensity of the light extinguished in the analyzer by $MP$ $\left(OM : MP :: \overline{OL}^2 : \overline{PL}^2\right)$. The intensities $OM$ and $OP$ are equal when the planes $CD$ and $AB$ coincide (parallel prisms); the intensity $OM$ is zero when the planes $CD$ and $AB$ are perpendicular (crossed prisms).

The construction and principle of the simplest form of polariscope can now be understood from the following diagram (Fig. 50). $P$ is the polarizer consisting of a stationary Nicol and $A$ is the analyzer consisting of a movable Nicol mounted in a revolving sleeve; the angular

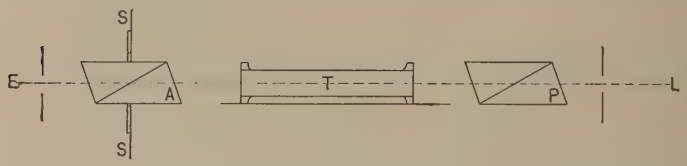



Fig. 50. — Showing arrangement of parts in a simple polariscope.

rotation of $A$ is measured upon a graduated scale $S$. $L$ is the source of monochromatic light which passes through the instrument to the eye of the observer at $E$. We will suppose the Nicol $A$ to be crossed with reference to $P$, the point of light extinction marking the zero point on the scale $S$. If a tube $T$ filled with a solution of some optically active substance, such as cane sugar, be now placed between $P$ and $A$, the plane of polarized light emergent from $P$ will be rotated from its original position and the light will no longer be entirely extinguished in $A$. By rotating the analyzer until its axial plane is perpendicular to the vibration plane of the light emergent from $T$, the point of extinction is again reached. The angular rotation of the solution in $T$ is then determined upon the graduated scale. By continuing the revolution of the analyzer, light will again emerge from the latter, to become reëxtinguished at a point 180 degrees from the first reading. Owing to the fact that light rays of different wave lengths are rotated to a different extent by optically active substances (a phenomenon known as rotation dispersion), it is necessary that the light used in this type of polariscope be monochromatic.

**Biot's Polariscope.** — The original polariscope of Biot* (Fig. 51), constructed in 1840, had an adjustable mirror ($M$) of black glass for

* Ann. chim. phys. [2], **74**, 401 (1840).

the polarizer and a modified prism of calc spar for the analyzer ($A$). The essential features of this early instrument are still retained in modern polarimeters, although in a greatly modified form.

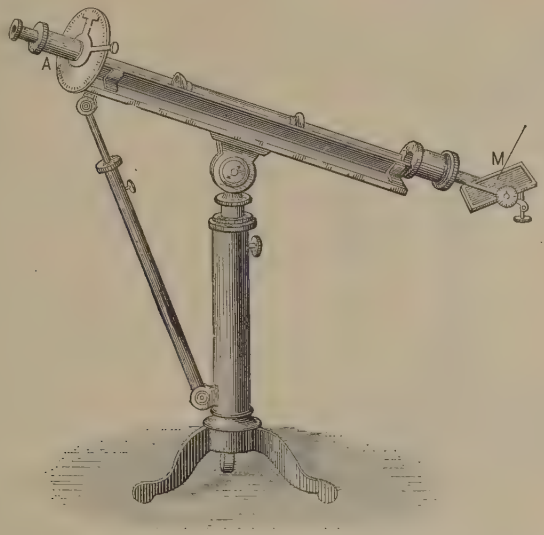



Fig. 51. — Biot's polariscope.

**Mitscherlich's Polariscope.** — Mitscherlich* in 1844 modified the Biot apparatus by discarding the polarizing mirror and arranging the optical parts of his instrument as shown in Fig. 50. In the Biot polariscope the end point was marked by total light extinction. But in the Mitscherlich apparatus a vertical black band with shaded margins marked the zero point. By rotating the analyzer gently to and fro until the vertical band appears exactly in the center of the field, a zero-point adjustment can be secured with a probable error of $\pm 6$ minutes. The Biot-Mitscherlich polariscope, with position of its optical parts, is shown in Fig. 52.

Sections of the circular scales used upon the Mitscherlich and other polarimeters for measuring the angular rotation of the plane of polarized light are shown in Figs. 53 and 54. The scale in Fig. 53 for a small polariscope indicates 0.1 degree and is immovable, the rotation being indicated by the position of the zero mark of the movable vernier $V$. In the illustration the zero mark of the vernier lies between the

* "Lehrbuch der Chemie" (1844), **1**, 361.

2-degree and 3-degree division of the scale; to obtain the fractions of a degree, one proceeds from the zero mark of the vernier and, moving upward along the divisions of the main scale, comes finally to a division which exactly coincides with one of the divisions of the vernier. In the illustration this vernier division is 0.4, which, added to the reading on the main scale, makes the angular rotation 2.4 degrees. For the larger polariscopes indicating 0.01 degree the main scale is movable, the circular rim divided into 0.25 degree rotating against the fixed vernier, which gives the readings to 0.01 degree. In the illustration (Fig. 54) the zero of the vernier falls between 13.50 degrees and 13.75 degrees; the 0.18 mark of the vernier is in coincidence with a division on the main scale. $13.50° + 0.18° = 13.68°$, which is the angular rotation indicated.

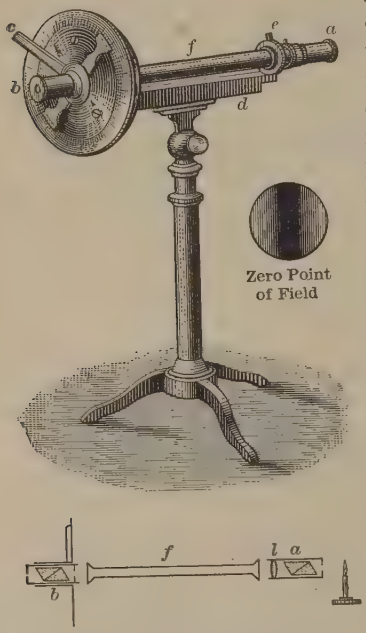



Fig. 52. — The Biot-Mitscherlich polariscope.

$a$ = position of polarizer
$b$ = position of analyzer
$c$ = lever for rotating analyzer
$l$ = condensing lens.

**Robiquet's Polariscope.** — Robiquet increased the sensibility of the Biot-Mitscherlich polariscope by introducing a Soleil double quartz plate as the end-point device. The general appearance of this instrument, with position of optical parts, is shown in Fig. 55.

*Principle of the Soleil Double Quartz Plate.* — The Soleil double quartz plate consists of two plates of quartz of equal thickness, one of which rotates the plane of polarized light to the right and the other to the left. The plates, which are cut perpendicular to the optical axis of the crystal, are cemented together at their edges and carefully ground and polished. If white polarized light pass through such a plate, the rays of different wave length and color will be rotated to a different degree (rotation dispersion), the rays of less wave length being rotated the most. For a piece of quartz 1 mm. thick, cut as above described, the rotation will be 15.75 degrees for the red $B$ ray, 21.72 degrees for the yellow $D$ ray of sodium, and 32.76 degrees for

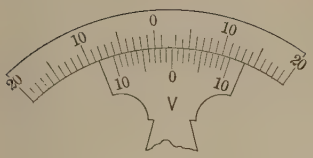



Fig. 53

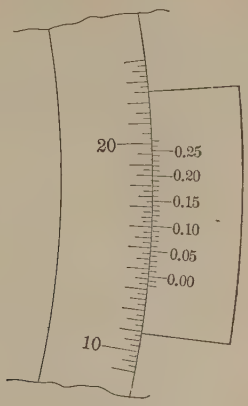



Fig. 54

Sections of circular scales of polariscopes.

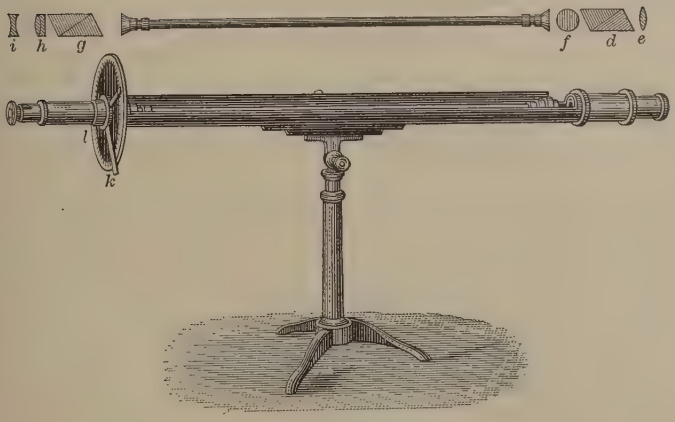



Fig. 55. — Robiquet's polariscope.

$d$ = polarizer
$e$ = condensing lens
$f$ = Soleil double quartz plate
$g$ = analyzer
$h$–$i$ = telescope
$k$ = lever for rotating analyzer.

the blue *F* ray. For the average ray in the middle of the yellow spectrum the rotation is 24 degrees. The thickness of the Soleil plate is so chosen that this average yellow ray is extinguished in the analyzer. This corresponds to a rotation of 90 degrees, or to a thickness of 3.75 mm. (90° ÷ 24 = 3.75) for the double plate, when the end point is taken for parallel Nicols. If a plate of the above description be inserted between two parallel Nicols and examined with white light, the color of the two halves will be of a uniform rose color, the blending of the spectral colors minus the yellow. The relationship of the angular rotations for red, yellow, and blue in the two halves of a 3.75 mm. plate at the transition point may be seen from Fig. 56. By rotating the analyzer to the right or left the uniform rose color of the plate will change, one-half to blue and the other to red, or vice versa. If a solution of an optical active substance be placed in the tube before the analyzer, the equilibrium in color of the transition tint will be destroyed and the two halves of the field will be differently colored. Rotating the analyzer to the point where the transition tint is again produced will give the angular rotation of the solution.

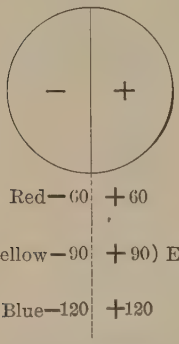



Fig. 56. — Showing principle of Soleil double quartz plate.

The Robiquet polariscope, which has a sensibility of about ± 4 minutes, is of course only adapted for white light. The rotation angle ($\alpha$) of a substance for extinction of the mean yellow ray was expressed by Biot as $\alpha_j$ ($j$ = French, *jaune;* yellow). The fact that the point $j$ corresponds to no well-defined line of the spectrum makes it a difficult one to verify, and some confusion has resulted from this cause. Landolt gives for 1 mm. quartz, $\alpha_j = 24.5$ degrees instead of 24 degrees. The value $\alpha_j$ is always greater than $\alpha_D$ (the rotation angle for the *D* ray of sodium). The relationship given by Landolt is $\alpha_j = \frac{24.5}{21.72}\alpha_D = 1.128\,\alpha_D$; using the value 24 degrees $\alpha_j = 1.105\,\alpha_D$. Many authorities employ the factor 1.111.

In the examination of colored solutions, the transition tint of the Soleil double plate is affected to such a degree that a considerable error is introduced in the observation. The use of this end-point device is valueless for the color-blind. For these reasons the transition-tint polariscopes are at present but little used.

**Jellet's Half-shadow Polariscope.** — Efforts to obtain a polarization apparatus which would be free from the defects of those previously named led Jellet* in 1860 to the construction of the first half-shadow polariscope. In this type of end-point adjustment, which can be secured in a variety of ways, the field of vision is divided into two or more parts, which at the zero position of the analyzer have a uniform shade. Rotating the analyzer to the right will cause one section of the field to become darker and the other lighter; rotation to the left will produce the opposite effect.

The half-shadow device of Jellet consists of a rhombohedron of calc spar with its ends cut square and bisected lengthwise by a plane forming a small angle with the axial plane of the prism; the two halves are then cemented together in the reversed position, the result being that the axial planes of each part are no longer parallel but are tilted toward one another at a slight angle. This reunited prism, placed between the polarizer and analyzer with its line of union bisecting the field, causes the planes of vibration of light proceeding from the polarizer to be slightly inclined towards one another in each half of the field. Rotating the analyzer until it is crossed with the polarizer will not produce extinction, but a uniform shadow or penumbra whose depth will depend upon the inclination of the axial planes in the two halves of the Jellet prism.

**Jellet-Cornu Prism.** — The Jellet polarizer was modified by Cornu† by taking an ordinary Nicol prism and dividing it lengthwise by a plane passing through the shorter diagonal of the end. A small wedge-shaped section is then removed from each cut surface and the two halves reunited (see Figs. 57 and 58). This "split" or "twin" prism combines the effect of an ordinary Nicol and Jellet prism.

The Jellet-Cornu prism was still further simplified by bisecting only one-half of the Nicol prism in the way described. The three pieces are then cemented together and the prism squared and mounted, with the split half turned toward the analyzer. This form of prism, sometimes called the Schmidt and Haensch polarizer, was formerly much used in the construction of half-shadow saccharimeters.‡

The principle of the half-shadow device of Jellet and its modifications may be seen from Fig. 59.

Let *GO* and *HO* represent the directions of the axial planes in each half of the Jellet prism, forming with each other the angle *GOH* (the

* Rep. Brit. Assoc., **29,** 13 (1860).

† Bull. soc. chim. [2], **14,** 140 (1870).

‡ Landolt, "Das optische Drehungsvermögen" (1898), p. 307.

half-shadow angle designated by $\alpha$ and made usually not to exceed 10 degrees). It will be seen that with the axial plane of the analyzer perpendicular to *PO* the light from the polarizer will not be completely extinguished in the analyzer; a small amount of light will emerge

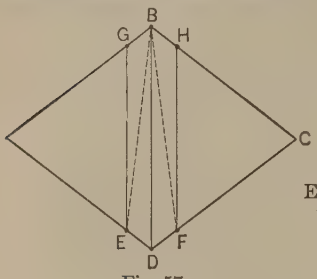



Fig. 57

End of Nicol prism before and after splitting.

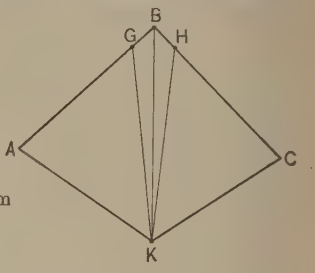



Fig. 58.

Showing construction of a Jellet-Cornu prism.

*BDE* and *BDF*, wedge sections removed.
*GE* and *HF*, directions of axial plane before cutting.
*GK* and *HK*, directions of axial planes after uniting cut surfaces.

from each half of the field proportional to the amplitudes *OM* and *ON* (see Fig. 49). The equality of light in the two divisions of the field constitutes the end point. By rotating the analyzer to the position *A'L'* perpendicular to *HO*, the light in the right half of the field will be

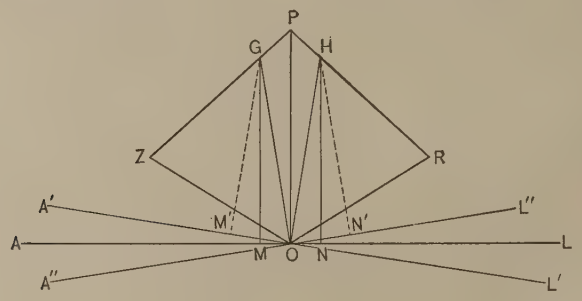



Fig. 59. — Illustrating principle of Jellet's half-shadow polariscope.

completely extinguished, and that in the left half will be increased from *OM* to *OM'*; similarly, with *A''L''* perpendicular to *GO* the light in the left half of the field is extinguished and that in the right half increased from *ON* to *ON'*; it is evident from the above that the half-shadow angle *GOH* can be measured by the angle *A'OA''* through which

the analyzer is rotated between the points of extinction in the two halves of the field. (For appearance of field at the several points see Fig. 61.)

There are several types of polariscopes which use the Jellet-Cornu polarizer for an end point. All of these have the advantage that they can be used with either mixed or homogeneous light, but the disadvantage that the half-shadow angle is fixed and cannot be changed to suit the requirements demanded by different kinds of work. The sensibility of the instrument to slight changes of rotation becomes greater as the half-shadow angle of the polarizer is made smaller; but, on the other hand, the loss of light at the end point produced by decreasing the inclination of the planes in the two halves of the field lessens the usefulness of the instrument in polarizing dark-colored solutions.

**Laurent's Half-shadow Apparatus.** — To overcome the last-named defect of the Jellet-Cornu polarizer, Laurent * in 1877 contrived an end-point device in which the half-shadow angle could be changed to suit varied requirements. The Laurent polariscope has the ordinary arrangement of Nicol prisms for polarizer and analyzer, the only difference being that the polarizer is attached to a small lever by which it can be rotated through a small angle to the right or left. The essential part of the end-point device is a thin plate of quartz cut perfectly plane and exactly parallel to its optical axis. This plate, which must be of specially prepared thickness, is mounted upon glass in such a way that it covers one-half of the field of vision. The rays of light from the polarizer on entering the plate are resolved into two components, one (the ordinary) vibrating in the plane of the optical axis, and the other (the extraordinary) in a plane perpendicular thereto. The extraordinary component, being less refracted, is transmitted more rapidly, and the thickness of the quartz plate is so regulated that when the two components emerge, the extraordinary one is in advance of the ordinary by half a wave length. The thickness of the plate depends upon the wave length $\lambda$ of the light, which must necessarily be homogeneous. The component rays which emerge from the quartz plate with half a wave length's (or uneven multiple thereof) difference in vibration are resolved by the analyzer into light which at the end point is of the same amplitude and intensity as that in the uncovered half of the field (the loss of light in the quartz plate by reflection and absorption being negligible). The two planes of vibration, which are inclined towards each other equally and symmetrically with reference to the optical axis of the plate, form the angle of the half shadow. The

* Dingler's Polytech. Jour., **223**, 608 (1877).

principle of the Laurent plate can be better understood from the following diagram (Fig. 60).

Let $LMNK$ represent the quartz plate with the edge $MK$ bisecting the circular field, $MK$ being assumed for convenience to coincide with the optical axis of the plate. Let $AA'$ represent the plane of the analyzer at the end point and $PP'$ the plane of the polarizer, the latter being set at the angle $POM$ with the optical axis $MK$. Lay off $OB$ as

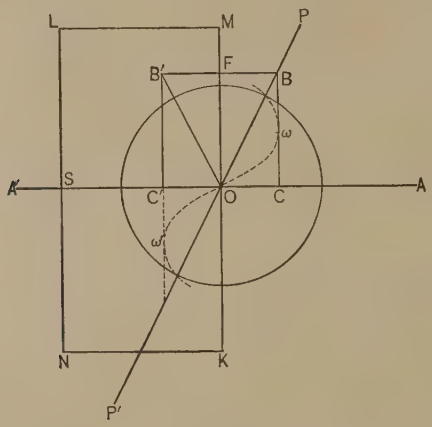



Fig. 60. — Showing principle of Laurent's half-wave plate.

the amplitude of the homogeneous light emerging from the polarizer and draw $BC \perp AA'$, then $OC$ will represent the amplitude of the light emergent from the analyzer for the uncovered half of the field (Fig. 49). The light of amplitude $OB$ upon entering the quartz plate is resolved into two components, one of which $OF$ (the ordinary ray) vibrates in the plane of the optical axis $MK$, and the other $OC$ (the extraordinary ray) vibrates in the plane $OS \perp MK$. The quartz plate is of such thickness that the extraordinary component entering at the phase $\omega$ is accelerated in its passage one-half wave length and emerges at the opposite phase $\omega'$. The amplitude $OC'$ being equal to $OC$, the resultant $OB'$, between $OC'$ and $OF$, is equal to $OB$, and the angle $B'OM$ equal to the angle $BOM$, the two together being the angle of the half-shadow. The light emergent from the analyzer in both halves of the field will therefore be equal in amplitude and intensity for any angle at which $PP'$ may be set with reference to $MK$. On rotating the analyzer from its position, the equilibrium in shade between the two halves will

be destroyed (Fig. 61),* the effect being the same as that described under Fig. 59.

The Laurent polariscope, which is the standard instrument in France, has the great advantage, over other forms, of adjustable sensibility without change in zero point, but the great disadvantage of being adapted to only monochromatic light. It cannot be used with

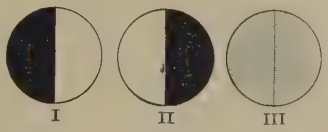



Fig. 61. — Showing divisions of double field of a half-shadow polariscope.

I, analyzer crossed with left half of field;
II, analyzer crossed with right half of field;
III, end point.

white light except when adapted to bichromate filtered light for a quartz wedge saccharimeter. With intense illumination and a small half-shadow angle (the conditions of greatest sensibility for all half-shadow instruments), the average error of observation according to Landolt is less than 1 minute.

*Concentric Half-wave Plate.*—Pellin has modified the Laurent polariscope by using a half-wave plate of quartz cut in circular or annular form. The field of vision is in this way divided concentrically as shown in Figs. 62 and 63.* While the concentric field may secure a more correct

Fig. 62

Concentric double field.

Fig. 63

Concentric triple field.

alignment of the eye with the optical axis of the polariscope, it is much more fatiguing to the eye than the ordinary bisected field. The principle of the concentric half-wave plate is the same as that of the Laurent plate.

* In Figs. 61, 62, 63, and 67b the dividing lines of the fields at end point are much intensified. With a properly adjusted instrument the dividing lines completely disappear at end point leaving a plain disk of uniform shade.

**Lippich's Half-shadow Polarimeter.**—In 1880 Lippich* devised a form of polarizer which combines the advantages of adjustable half-shadow and of adaptability to all kinds of light. The Lippich polarizer consists of two Nicol prisms, one large Nicol, which can be rotated about its long axis according to the needs of sensibility, and one smaller Nicol, known as the "half-prism," which is mounted in front of the large Nicol so as to cover one-half of the field. The half-prism is slightly tilted so that its inner vertical edge forms a sharply dividing line, which can easily be focused by the eyepiece of the instrument (Fig. 64).

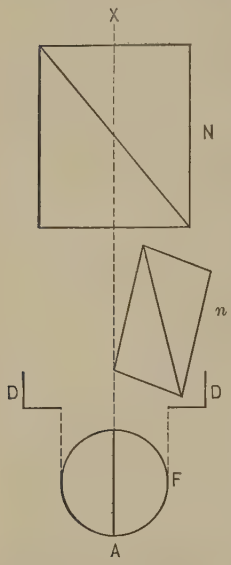



Fig. 64.—Showing construction of a Lippich polarizer for double field.

$N$ = large Nicol;
$n$ = small Nicol or "half prism;"
$D$ = margin of diaphragm;
$F$ = projection of field.

The principle of the Lippich polarizer can be understood by referring to the opposite diagram (Fig. 65):

Let $OP$ be the plane of the large Nicol and $OH$ the plane of the half-prism, the included angle $POH$ being that of the half-shadow $\alpha$. Let $OB =$ the amplitude of the light emergent from the large Nicol. Draw $BG \perp OH$. Then $OG$ will represent the amplitude of the light emergent from the half-prism. It can readily be seen that with a loss of a part of the light in the half-prism the amplitudes $OC'$ and $OD'$ in the two halves of the field do not agree when the perpendicular $OA'$ to the plane of the analyzer bisects the half-shadow $\alpha$. By rotating the analyzer slightly from $L'M'$ to $LM$ the amplitudes $OC$ and $OD$ are made equal, in which position the perpendicular $OA$ no longer bisects $\alpha$. The angle $\delta$ which the perpendicular $OA$ makes with the bisector $OA'$ will vary according to the size of the half-shadow angle $\alpha$. The Lippich polarizer is therefore not symmetrical, which is a disadvantage, since by changing the half-shadow $\alpha$ to vary the sensibility there is also a change in the zero point of the analyzer. The latter must accordingly be readjusted for each change in sensibility.

The relation of intensities in the light emerging from the large and small prisms of the Lippich polarizer is found as follows:

* Z. Instrument., **2**, 167; **14**, 326.

$\frac{OG}{OB} = \cos \angle BOG = \cos \alpha$. If $I$ and $I'$ are the intensities for the large and small prisms respectively, then

$$\frac{I'}{I} = \frac{\overline{OG}^2}{\overline{OB}^2} = \cos^2 \alpha \quad \text{and} \quad I' = I \cos^2 \alpha. \tag{1}$$

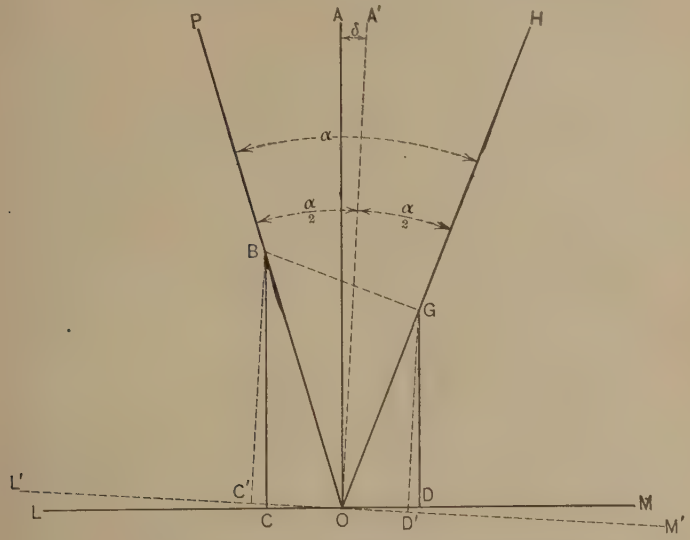



Fig. 65. — Illustrating principle of Lippich polarizer.

The relation between the angle of the half-shadow $\alpha$ and that of the change in zero point $\delta$ may be calculated as follows: When the two halves of the field are matched the amplitudes $OC = OD$ and the intensities $\overline{OC}^2 = \overline{OD}^2$.

$$\frac{OC}{OB} = \sin \angle CBO = \sin \angle POA = \sin\left(\frac{\alpha}{2} - \delta\right).$$

$$\frac{OD}{OG} = \sin OGD = \sin \angle HOA = \sin\left(\frac{\alpha}{2} + \delta\right).$$

$$\frac{\overline{OC}^2}{\overline{OB}^2} = \sin^2\left(\frac{\alpha}{2} - \delta\right). \tag{2}$$

$$\frac{\overline{OD}^2}{\overline{OG}^2} = \sin^2\left(\frac{\alpha}{2} + \delta\right). \tag{3}$$

Substituting $I$ and $I'$ for $\overline{OB}^2$ and $\overline{OG}^2$, we obtain

$$\overline{OC}^2 = \sin^2\left(\frac{\alpha}{2} - \delta\right) I.$$

$$\overline{OD}^2 = \sin^2\left(\frac{\alpha}{2} + \delta\right) I'; \text{ since } \overline{OC}^2 = \overline{OD}^2 \text{ for the matched field, we obtain}$$

$$\sin^2\left(\frac{\alpha}{2} - \delta\right) I = \sin^2\left(\frac{\alpha}{2} + \delta\right) I'. \tag{4}$$

$$\sin^2\left(\frac{\alpha}{2} - \delta\right) = \sin^2\left(\frac{\alpha}{2} + \delta\right)\frac{I'}{I} = \sin^2\left(\frac{\alpha}{2} + \delta\right)\cos^2\alpha. \tag{5}$$

$$\sin\frac{\alpha}{2}\cos\delta - \cos\frac{\alpha}{2}\sin\delta = \sin\frac{\alpha}{2}\cos\delta\cos\alpha + \cos\frac{\alpha}{2}\sin\delta\cos\alpha.$$

Dividing by $\cos\frac{\alpha}{2}\cos\delta$, we obtain

$$\tan\frac{\alpha}{2} - \tan\delta = \tan\frac{\alpha}{2}\cos\alpha + \tan\delta\cos\alpha.$$

$$\tan\delta = \tan\frac{\alpha}{2}\,\frac{1 - \cos\alpha}{1 + \cos\alpha} = \tan^3\frac{\alpha}{2}. \tag{6}$$

In the above calculation only the light extinguished in the small Nicol has been considered. There are other factors, however, which must be taken into account in the calculation of the true zero-point correction. Schönrock* has shown that 7.5 per cent of the light is lost by reflection from the surface of the small Nicol, and that this amount is increased to 8 per cent or more by the loss through absorption. Equation 1 for intensity would then become

$$I' = I\cos^2\alpha\sqrt{0.92}. \tag{7}$$

The value of $\delta$ thus modified would be expressed by

$$\tan\delta = \frac{1 - \cos\alpha\sqrt{0.92}}{1 + \cos\alpha\sqrt{0.92}}\tan\frac{\alpha}{2}. \tag{8}$$

Bates† has shown, however, that a part of the light lost by reflection from the sides of the small Nicol is again restored in the analyzer, and that when all factors such as depolarization, size, shape, and inclination of the small prism, etc., are taken into account the true value of $\delta$ is between those calculated by equations 6 and 8, the exact figure depending upon the construction of each individual Lippich system.

Apart from the disadvantage that the zero point must be corrected

* Z. Ver. Deut. Zuckerind., **58**, 111.

† Ibid., **58**, 821.

for changes in sensibility, the Lippich polarizer is the best for general use and the one most sensitive to minute changes in rotation. The average error of adjustment, according to Landolt, with bright illumination and a half-shadow angle of 1 degree, is only about 15 seconds (0.004 degree).

*Lippich Polarizer with Triple Field.* — The sensibility of the Lippich polarizer has been almost doubled by using two half-prisms in place of one, the system being so arranged that the field of vision is divided into three parts (Figs. 66 and 67). The principle of the triple field can be understood by referring to Fig. 67a.

Let $AC$, $ac$, and $a'c'$ represent planes of the large Nicol $N$, and $ab$ and $a'b'$ planes of the half-prisms $n$ and $n'$ respectively. It will be seen that $ab$ and $a'b'$ must be perfectly parallel in order that the half-shadow angles $\alpha$ and $\alpha'$ be equal for both half-prisms, an absolute essential if perfectly uniform illumination is to be obtained at the end point. It sometimes happens that the two half-prisms get out of parallelism through jarring of the instrument or expansion and contraction of the mountings. There will then be two end points for the half-shadow, according to which side the middle of the field is made to agree. The observer is then obliged either to take but one of these end points, which is equivalent to reducing the instrument to an imperfect double field, or else to readjust the planes of the half-prisms to parallelism, a most delicate as well as time-consuming operation. For instruments requiring constant use the increase in sensibility of the triple field can hardly be said to offset the increased sensitiveness of the polarizer to disarrangement. The more simple double-field end-point device is much to be preferred for ordinary laboratory conditions.*

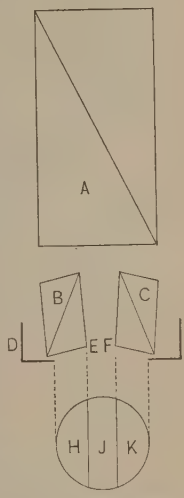



Fig. 66. — Showing construction of Lippich polarizer for triple field.

*A*, large Nicol;
*B* and *C*, small half-prisms;
*D*, margin of diaphragm;
*E* and *F*, inner edges of half-prism which form the divisions *H*, *J*, and *K* of the triple field.

*Lippich Polarizer with Quadruple Field.* — Lummer† has constructed a polarizer with quadruple field (Fig. 68) by placing before the larger

* Many chemists wrongly use the expressions half-shade and triple-shade in place of the terms double field and triple field. The term half-shade or half-shadow, (German, *Halbschatten;* French, *pénombre*), refers to the depth of shade in the field at the end point and not to the division of the field. The expression triple shade is meaningless.

† Z. Instrument., **16**, 209.

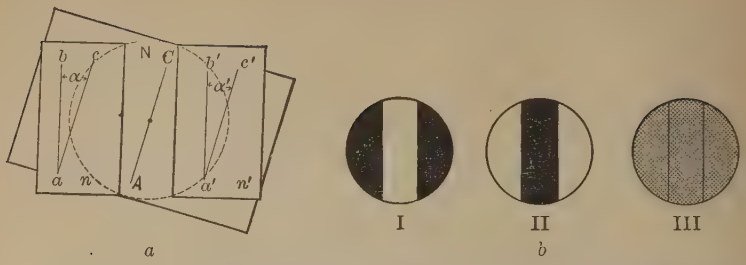



Fig. 67. — Illustrating principle of Lippich polarizer for triple field.
I, analyzer crossed with outer divisions of field;
II, analyzer crossed with inner divisions of field;
III, end point.

Nicol $A$ one large half-prism $B$, and before the latter two smaller half-prisms $C$ and $D$. The increased complication of this form of polarizer has prevented its general introduction.

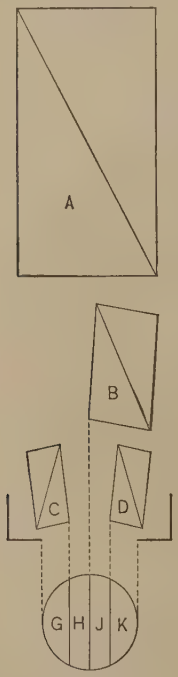



Fig. 68.— Showing construction of Lippich polarizer for quadruple field.

**Wild's Polaristrobometer.** — Another form of polarizing apparatus, whose peculiarities of construction place it in a class by itself, is the polaristrobometer invented by Wild* in 1864. In this instrument, shown in Fig. 69, the polarizer ($f$) is attached to a divided circle, $K$, both being rotated by a rod and pinion from the screw $C$ around the longitudinal axis of the Nicol prism. The end-point device placed at $e$ consists of a Savart double plate made up of two sections of calc spar each 3 mm. thick, cut at an angle of 45 degrees to the optical axis of the crystal, and cemented together so that their principal sections cross at right angles. A diaphragm $c$ with cross threads is placed in the focus of the objective lens $d$ of the telescope. The analyzer at $a$ is stationary, being usually mounted with its principal section horizontal and forming an angle of 45 degrees with the crossed sections of the Savart plate.

* "Ueber ein neues Polaristrobometer," Bern, 1865.

To determine the zero point of the polaristrobometer, which is first illuminated at *D* with a sodium flame, a tube of water is placed in the instrument and the ocular of the telescope focused sharply upon the cross threads; the field, except near the end point, consists of a series of dark horizontal parallel bands, the so-called interference fringes, which

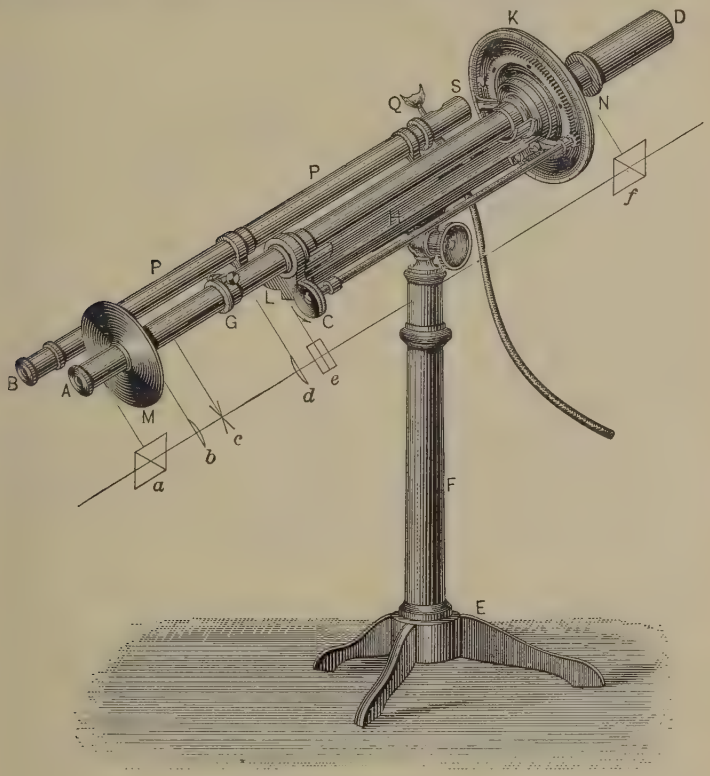



Fig. 69. — Wild's polaristrobometer.

upon rotation of the polarizer increase and decrease in intensity; at certain points of rotation the bands gradually become paler until, at the maximum point of brightness, they are suddenly extinguished in the center of the field, leaving only a slightly shaded border at each edge (see Figs. 70 and 71). The point at which the shaded borders and the extinguished part of the field are symmetrically distributed with reference to the cross threads constitutes the end point. In this

position the plane of the polarizer is parallel with one of the crossed planes of the Savart plate, so that the end point reoccurs every 90 degrees. In case the extinguished part of the fringes is too wide for accurate adjustment, the intensity of the light should be diminished until the borders of the fringes are brought sufficiently close to the reticule. The fringes have usually a different appearance at each of the end points, and also with colored solutions, so that a beginner must familiarize himself with the various characters of the field before making

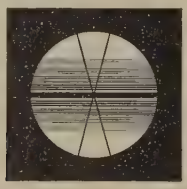


Fig. 70

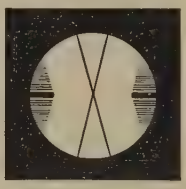


Fig. 71

Showing field of Wild's polaristrobometer.

Fig. 70. — Interference fringes before end point.
Fig. 71. — Interference fringes at end point.

readings. In case the zero points of the scale and vernier do not coincide at the end point, the deviation may be noted and applied to the readings as a correction, or else they may be set at zero and the instrument brought into adjustment by gently turning the screw $G$ until the proper end point is secured.

If the polarizer is set at one of the four zero points and a tube of sucrose solution be placed in the trough, the interference fringes will reappear. The polarizer must then be rotated to the left (opposite to the rotation of the sugar solution) until the fringes again disappear. The angular displacement of the polarizer to the left gives the angular rotation of the sucrose solution to the right. The readings are made through a telescope $P$ which is focused upon the fixed vernier $J$; the latter is illuminated by a flame at $Q$. The average error of adjustment according to Landolt is about $\pm 3$ minutes.

The divisions of the scale upon the Wild polaristrobometer are made usually in both circular degrees and in degrees of a sugar scale giving percentages of sucrose. The sugar scale is constructed by dividing 53.134 circular degrees into 400 equal parts. Each of these sugar divisions corresponds to the rotation of 1 gm. of sucrose dissolved to 1000 c.c. and polarized in a 200-mm. tube; 10 gms. of pure sucrose dis-

solved to 100 c.c. will indicate the 100-degree point of Wild's scale, 20 gms. sucrose dissolved to 100 c.c. will indicate the 200-degree point, 30 gms. the 300-degree point, and 40 gms. the 400-degree point. The normal weight of the sugar scale of the Wild polaristrobometer can therefore be varied according to the concentration of the product to be examined, the readings obtained with the 20-gm., 30-gm., and 40-gm. normal weights being divided by 2 or 3 or 4, as the case may be.

The Wild polaristrobometer, although formerly used in many European laboratories, finds at present but limited application in technical sugar analysis.

### Description of Standard Modern Polarimeters

The concluding parts of this chapter will be devoted to descriptions of a few standard forms of modern polarimeters.

**Laurent's Polarimeter.** — As a type of instrument of French manufacture the Laurent polarimeter is shown in Fig. 72.

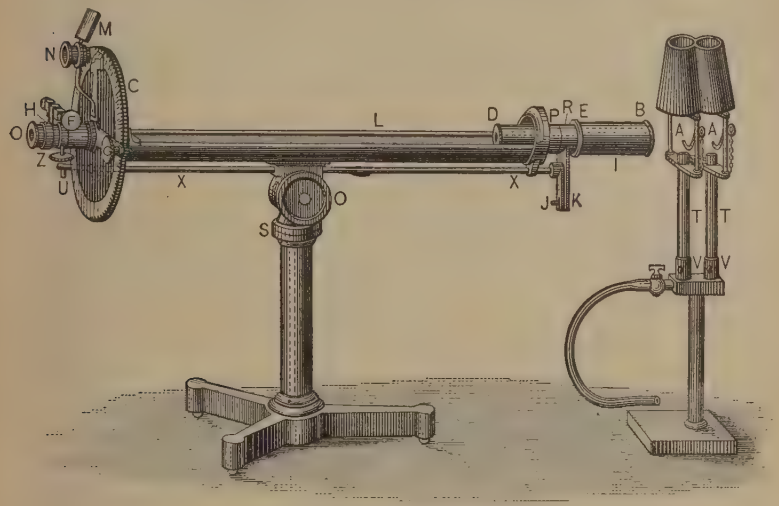



Fig. 72. — Laurent's polarimeter.

*A*. A duplex Laurent sodium burner placed 200 mm. from *B*.
*B*. Illuminating lens.
*C*. Quadrant whose outer circle is divided into circular degrees and whose inner circle is divided into sugar degrees.
*D*. Diaphragm containing half-wave plate of quartz.
*E*. Light filter consisting of a crystal of potassium bichromate.

*F*. Screw for adjustment of zero point.

*G*. Geared screw for rotating the analyzer and the arm supporting the verniers. The upper vernier on the right is for reading circular degrees and the lower vernier upon the left for reading sugar degrees.

*L*. Bronze trough 600 mm. long for holding observation tubes.

*M*. Mirror for illuminating scale.

*N*. Magnifying glass for reading scale.

*R*. Tube section containing polarizer; the latter can be moved through a small angle by the arm *K*, which is moved by the crank *J* through the rod *X* by means of the lever *U*. If the solution to be examined is but little colored, the lever *U* is raised, which decreases the half-shadow angle. With colored solutions *U* is lowered until the half-shadow is increased to the point of greatest sensibility. The zero point should be redetermined after each change in the position of the polarizer.

The 100-degree point of the sugar scale of the Laurent polarimeter corresponds to an angular rotation of 21.67 degrees (21° 40′), which is the value given by French authorities to the angular rotation of the 1 mm. thick plate of quartz cut perpendicular to the optical axis (see page 112). The normal weight of sucrose corresponding to this rotation is given as 16.29 gms. dissolved to 100 c.c. and polarized in the 200-mm. tube. The sugar scale extends 400 divisions to the right and 200 divisions to the left, thus giving ample range for polarizing all dextro- and levo-rotatory sugars. If desired, the sugar scale of the Laurent polarimeter is adjusted according to the so-called International saccharimetric scale of 20 gms. The value of the 100-degree division of the International scale in circular degrees would equal $\frac{21.67 \times 20}{16.29} = 26.605$ degrees; this is a trifle more than the circular value of the Wild 20-gm. scale, viz., 26.567 degrees, the difference being due presumably to the adoption of a slightly different standard value for the specific rotation of sucrose.

**Pellin's Polarimeter.** — Another type of French polariscope is the half-shadow polarimeter-saccharimeter made by Pellin, shown in Fig. 73. The polarizer of this instrument consists of a modified Jellet-Cornu prism; the half-shadow angle is therefore fixed. The division of the quadrant into circular and sugar degrees is identical with that of the Laurent polarimeter.

The Pellin polarimeter with variable half-shadow angle (Fig. 74) makes use of a half-wave plate of quartz for the end point, which is constructed for either divided or concentric fields. The arrangement of optical parts and method of manipulation are the same as in the Laurent polarimeter.

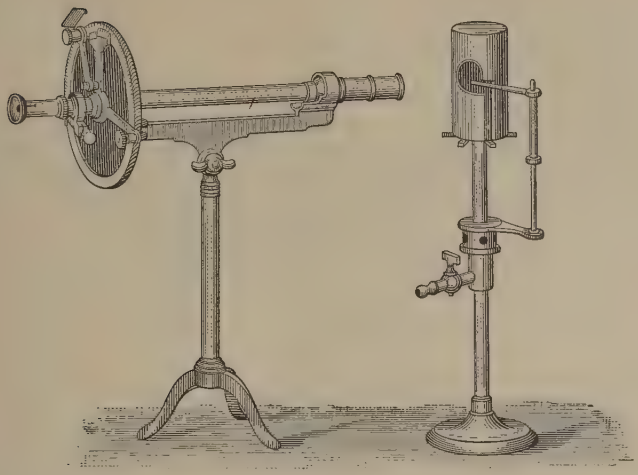


Fig. 73. — Pellin's polarimeter with Jellet-Cornu prism.

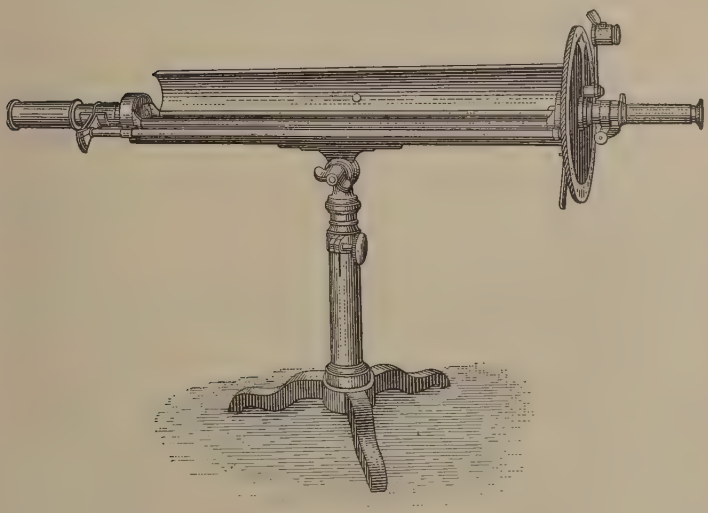


Fig. 74. — Pellin's polarimeter with half-wave plate.

**Lippich's Polarimeter.** — A simple form of Lippich's polarimeter adapted for general chemical use is shown in Fig. 75. Angular rotations can be measured with this instrument to about 0.015 degree.

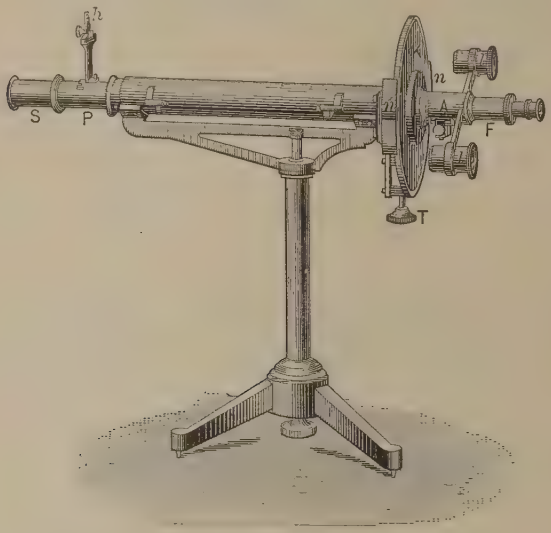



Fig. 75. — Simple form of Lippich's polarimeter.

*h*. Lever for moving large Nicol of polarizer and regulating sensibility. The half-shadow angle which is read by the scale can be varied from 0 degrees to 20 degrees.

*K*. Divided circle for measuring rotation. The circle with analyzer in *A* and telescope at *F* is rotated by the screw *T*. The readings of the scale are made on each side of the circle through the lenses *l*, which are focused upon the fixed verniers at *n*.

*P*. Location of Lippich polarizer.

*S*. Detachable end for holding light filter.

A form of the Lippich apparatus devised by Landolt for more general use is shown in Fig. 76. This instrument presents an advantage in that any form of tube or container may be used for holding the solution or substance to be polarized.

The trough *D* of the polariscope for holding ordinary tubes can be removed and the support *T* employed. The latter is raised or lowered by the screw *q* and moved laterally upon the tracks *c*. For polarizing materials in hot or cold condition, the apparatus *G*, consisting of a

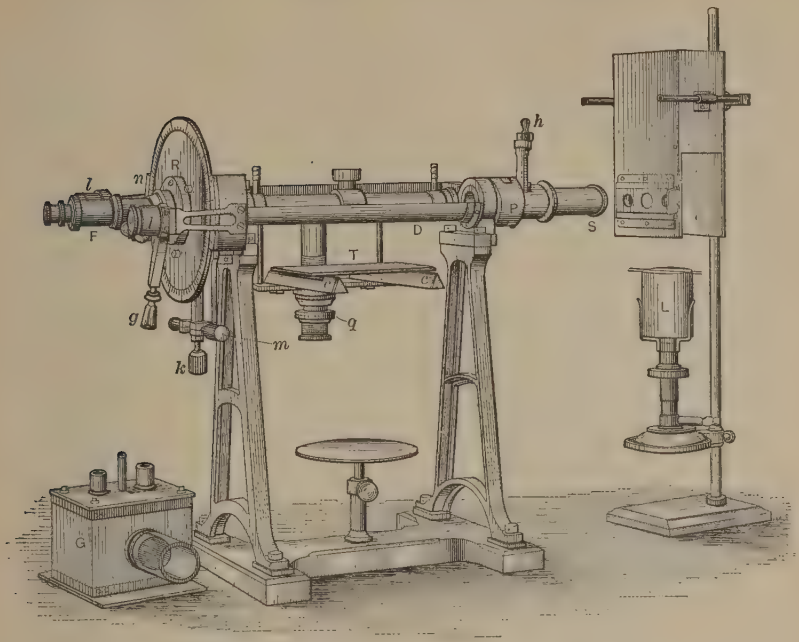



Fig. 76. — Landolt's polarimeter for general use.

*g*. Lever for rotating circle *R*; the final adjustment is made by means of the micrometer screw *m* after fixing the clamp *k*.

*P*. Position of Lippich polarizer with two half-prisms giving triple field.

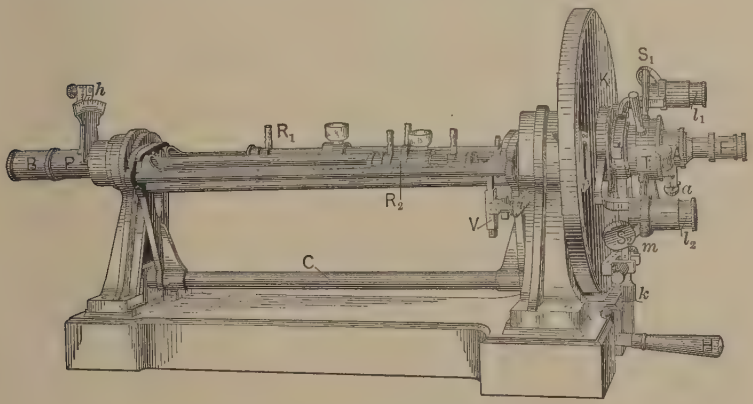



Fig. 77. — Large model Landolt polarimeter.

polariscope tube in an asbestos-jacketed bath, is employed. The plate $T$ is then removed and the bath placed directly upon the tracks $c$. The burner for heating the bath is placed upon the adjustable stand underneath. The center narrow tube projecting through the replaceable top of the bath receives the overflow from the observation tube; the other tubes serve for a thermometer and stirrer for the liquid of the bath. For polarizing at low temperature a cooling medium is used in the bath, in which case the ends of the observation tubes must be covered with desiccating caps to prevent condensation of moisture upon the cover glasses.

A type of more elaborate polarimeter, which can be read to 0.01 degree, is the large Landolt instrument shown in Fig. 77. The divided circle (driven by the wheel $T$ and micrometer screw $m$) is covered by a cap $K$. Small mirrors $S_1$ and $S_2$ reflect light from the observation lamp through openings in the cap to illuminate the scale. A feature of this instrument is the double trough by which different tubes of solution can be brought into the field by movement of the large lever $H$.

### Verification of Scale Reading of Polarimeters

The graduations of the divided circle upon a polarimeter should be verified by taking check readings at different points upon opposite sides of the disk. The division and mounting of the circle in the best instruments is made with great accuracy, and, unless the disk has been warped or bent, check readings on opposite sides of the circle will agree much closer than the observer can set the scale for a matched field.

Polariscope readings should always be verified upon the opposite scale. It is also well to reverse the circle 180 degrees and repeat the readings each way from the other side. By so doing the observer will have 4 sets of readings, the mean of which will practically eliminate all errors due to faulty scale division or eccentricity. The example on page 107 of readings made upon a sugar solution will illustrate the method.

The adjustment of the half-shadow angle is made to the point of greatest sensibility, the angle being small for light-colored solutions and larger for dark liquids. Since altering the half-shadow of the Lippich system produces a change in zero point (p. 95), the adjusting lever should never be disturbed during a set of observations. The analyzer, if desired, can be brought back to the 0 of the scale for any change in the half-shadow angle by means of a small regulating screw (shown at $a$, Fig. 77). The better method, however, is to establish the zero point upon the scale, as in the following example, and subtract this from the scale reading.

| | Zero point. | | Sugar solution. | | |
|---|---|---|---|---|---|
| | Right. | Left. | Right. | Left. | |
| Half-shadow angle = 6° | 3.07 | 183.07 | 29.30 | 209.295 | Temperature 20° C. |
| | 3.09 | 183.085 | 29.28 | 209.28 | |
| | 3.11 | 183.11 | 29.295 | 209.29 | |
| | 3.08 | 183.075 | 29.27 | 209.28 | |
| | 3.10 | 183.10 | 29.285 | 209.29 | |
| Average. | 3.09 | 183.088 | 29.286 | 209.287 | |
| | | | 3.090 | 183.088 | |
| | | | 26.196 | 26.199 | |
| Reversing the circle 180°. | 183.075 | 3.08 | 209.270 | 29.265 | Temperature 21° C. |
| | 183.10 | 3.10 | 209.285 | 29.28 | |
| | 183.08 | 3.085 | 209.28 | 29.28 | |
| | 183.09 | 3.09 | 209.27 | 29.27 | |
| | 183.09 | 3.095 | 209.285 | 29.285 | |
| | 183.087 | 3.090 | 209.278 | 29.276 | |
| | | | 183.087 | 3.090 | |
| | | | 26.191 | 26.186 | |

Average of 4 readings, 26.193° for 20.5° C.

# CHAPTER VI

## THEORY AND DESCRIPTION OF SACCHARIMETERS

While the instruments described in the previous chapter are adapted to the examination of all optically active substances, saccharimeters are designed solely for polarizing sugars. For convenience the scale expressing angular rotation is replaced upon the saccharimeter by one graduated according to the decimal system indicating percentages.

### The Quartz-wedge Compensation

Owing to the many difficulties and inconveniences connected with the use of sodium or other monochromatic light in practical work, the French physicist Soleil was led in 1848 to devise a means by which ordinary daylight or lamplight could be used for measuring the optical rotation of sugar solutions. This invention, known as the quartz-wedge compensation, is the characteristic feature of all saccharimeters.

In the quartz-wedge saccharimeter the polarizer and analyzer are both stationary; the rotation of the sugar solution is measured by shifting a wedge of optically active quartz between the solution and analyzer until the rotation of the wedge system at a certain thickness exactly neutralizes or compensates the rotation of the sugar solution. By means of a scale attached to the quartz wedge the rotation of the sugar in solution is measured in percentage.

The selection of quartz for compensation is based upon the fact that it has almost exactly the same rotation dispersion as cane sugar; i.e., a section of quartz and a cane-sugar solution of equal rotation for light of one wave length will have very nearly equal rotations for light of all other wave lengths (see Table XX). The small disturbances due to the slight difference in rotation dispersion between sugars and quartz are eliminated by a bichromate light filter.

**Single-wedge System.** — The quartz wedges used in the construction of saccharimeters are cut perpendicularly to the optical axis of the quartz crystal; they may be either of dextrorotatory or levorotatory quartz, the method of mounting the wedge depending upon the character of the rotation. This can be seen more clearly by inspecting the following diagrams (Fig. 78).

In diagram I, $A$ is a fixed plate of levorotatory quartz, and $B$ and $C$ two wedges of dextrorotatory quartz, of which $B$ is movable and $C$ stationary. The two wedges, which though of different size must have equal angular dimensions, may be considered to form together a single section with sides parallel to the plate $A$ and perpendicular to the axis of light through the instrument. The thickness of the two wedge sections can be increased or diminished by moving wedge $B$ to the right or left. At the zero point of the instrument the right rotation of

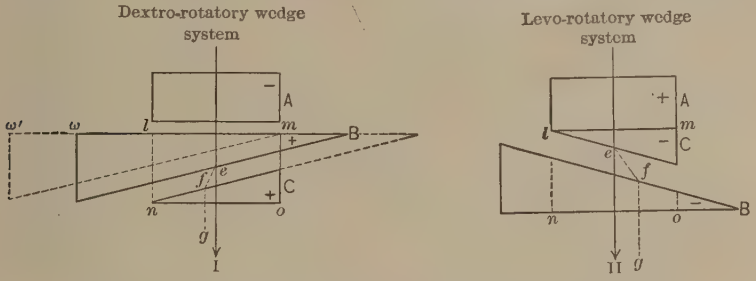



Fig. 78. Showing construction of single wedge quartz compensation.

the section *lmno* of the two-wedge system exactly neutralizes the left rotation of the quartz plate $A$. If a tube of dextrorotatory sugar solution be now placed in the instrument between the polarizer and the compensation plate $A$, the optical neutrality is destroyed, and it will be necessary to decrease the thickness of the two-wedge section by sliding $B$ from $\omega$ towards $\omega'$ until the excess of left rotation in $A$ over $B$ and $C$ exactly neutralizes the right rotation of the sugar solution. If the solution of sugar is left-rotating, it will be necessary to slide $B$ in the opposite direction until the excess of right rotation in $B$ and $C$ over $A$ equals the left rotation of the sugar. In a levorotatory wedge system (diagram II) the compensation plate $A$ is dextrorotatory and the wedges $B$ and $C$ levorotatory, the compensating motion of wedge $B$ being the reverse of that in diagram I.

Owing to the lateral refraction of light from the inclined surfaces of the wedges through the intervening air space (as shown by the dotted line *efg*), the planes of quartz are separated only just sufficiently to allow free movement of the parts without friction. The circumstance that the field is not exactly at the end point, when the thickness of the two-wedge section agrees with that of the compensating plate, is due to this lateral refraction. The shifting of zero point due to refraction depends upon the wave length of light; the difference in zero

point between red light of 760 $\mu\mu$ wave length and violet light of 396.8 $\mu\mu$ wave length was found by Schönrock to be 0.059 degree for the Ventzke sugar scale.

The scale of the saccharimeter is attached to the large or movable wedge, and is read by means of a vernier scale attached to a regulating screw. In case the zero marks of the two scales do not agree, when the two halves of the field correspond in shade, they can be brought into coincidence by shifting the vernier slightly to the right or left by means of a key which fits the regulating screw. The vernier is never to be moved except for making this adjustment, and when the two scales are once set has rarely to be disturbed. Owing to the inevitable slight fluctuations in the zero point of saccharimeters, it is best to correct the reading by the zero-point error and not to adjust the scale unless there be a persistent difference of the zero point in one direction greater than 0.1 degree. The method of reading the saccharimeter scale can be seen from Figs. 80 and 81.

**Double-wedge System.** — An elaboration of the quartz-wedge system just described is the double-wedge compensation introduced by Schmidt and Haensch. The arrangement of the parts in the double-wedge system is shown in Fig. 79.

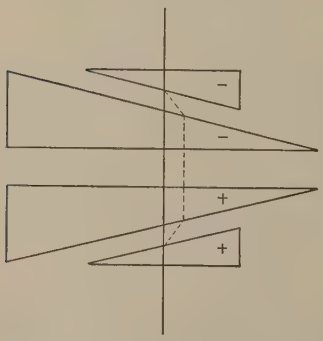



Fig. 79. — Showing construction of double wedge quartz compensation.

In the double-wedge system the compensation plate is lacking, this being supplied by one or the other of the pair of wedges, which are of opposite rotation. The smaller wedges $A$ and $D$ are stationary and the larger wedges $B$ and $C$ movable. $B$ and $C$ are usually mounted with their points in the same direction in order to equalize the refraction of the light rays in the air spaces between the inclined surfaces of quartz (as indicated by the dotted line); for this reason also the corresponding wedges of each system are made as near alike as possible. Each of the large wedges is provided with a scale. These may be read through the same telescope as upon the Schmidt and Haensch saccharimeter (Fig. 80), or by separate telescopes as in the Frič instruments (Fig. 81).

In using the double-wedge system for dextrorotatory substances, the scale $K$ (Fig. 80) is set at zero with its vernier, and the optical rota-

tion measured upon the scale $A$; for levorotatory solutions, $A$ is set at zero and the scale $K$ employed. An additional advantage of the double-wedge system consists in the fact that any reading obtained upon

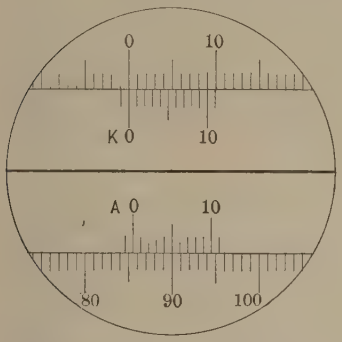



Fig. 80. — Scale of double wedge Schmidt and Haensch saccharimeter.
$K$, control scale;
$A$, working scale indicating 85.5 degrees Ventzke.

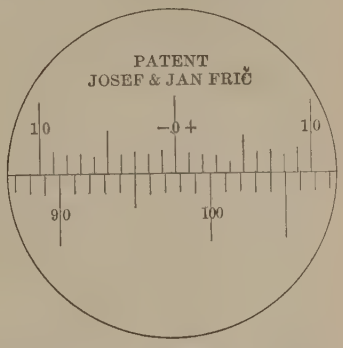



Fig. 81. — Scale of Frič saccharimeter with double vernier indicating 97.7 degrees Ventzke. (The division between scale and vernier is intensified; in reality no dividing line is seen.)

the working wedge can be immediately verified by removing the tube of solution and moving the control wedge to the point of compensation. The control wedge under such conditions gives the true reading directly, even though the working wedge have a zero-point correction.

| Zero-point determination. | | | Polarization of mat sugar. | | |
|---|---|---|---|---|---|
| Control-wedge scale. | Working-wedge scale. | Difference. | Control-wedge scale. | Working-wedge scale. | Difference. |
| 0.00 | 0.10 | +0.10 | 0.00 | 89.40 | 89.40 |
| 11.55 | 11.65 | +0.10 | 0.75 | 90.15 | 89.40 |
| 20.75 | 20.80 | +0.05 | 2.15 | 91.50 | 89.35 |
| 32.20 | 32.30 | +0.10 | 2.90 | 92.30 | 89.40 |
| 43.75 | 43.80 | +0.05 | 3.85 | 93.25 | 89.40 |
| 52.50 | 52.55 | +0.05 | 5.45 | 94.85 | 89.40 |
| 61.85 | 61.95 | +0.10 | 6.55 | 96.00 | 89.45 |
| 70.50 | 70.60 | +0.10 | 7.95 | 97.30 | 89.35 |
| 81.15 | 81.30 | +0.15 | 9.10 | 98.45 | 89.35 |
| 91.15 | 91.25 | +0.10 | 10.15 | 99.55 | 89.40 |
| Average zero point | | +0.09 | Average polarization uncorrected..... | | 89.39 |
| | | | Zero-point correction = | | 0.09 |
| | | | Corrected polarization = | | 89.30 |

*Zero-point Determination.* — The zero-point correction of the working wedge can be determined very accurately by taking check readings at different parts of the scale upon the control. By making polarizations in the same way, the local defects of scale or wedge will be almost wholly eliminated. The readings in this case are made without removing the tube, the difference between the two scales being the *uncorrected* polarization. The preceding table, giving the readings upon the working-wedge scale for various positions of the control, will illustrate the method.

### The Sugar Scale and Normal Weight of Saccharimeters

The 100-degree point of a saccharimeter scale is usually based upon the rotation of a definite weight (the so-called normal weight) of chemically pure sucrose dissolved in water to 100 c.c. at a specified temperature and polarized at the same temperature in a 200-mm. tube. The greatest confusion has prevailed in saccharimetry in the past, and unfortunately still prevails, not only as to the size of the normal weight of sugar to be taken for a specified scale, but also as to the conditions of volume and temperature under which this normal weight is to be polarized.

**French Sugar Scale.** — The 100-degree point of the sugar scale employed upon saccharimeters of French manufacture is based upon the rotation in sodium light of a plate of dextrorotatory quartz 1 mm. in thickness and cut exactly perpendicular to the optical axis. The choice of quartz as a standard proved to be unfortunate, for, owing either to mistakes of polarimetric measurement or to defects in the quartz (through natural imperfection or mistakes in cutting), the rotation of the 1-mm. plate has been given a different value from time to time, the results ranging from +20.98 degrees, the early figure of Biot, to +22.67 degrees. Most French authorities at present employ the value +21.67 degrees. The figure, regarded usually as the most exact, is that of Landolt, who, for spectral pure Na light of mean wave length 589.3 $\mu\mu$, found the value +21.723 degrees. The grams of sucrose necessary to give the same rotation in 100 c.c. as the 1-mm. quartz plate have also necessarily varied; over 20 different values have been assigned to this quantity, the amounts ranging from 16.000 gms. (Dubrunfaut) to 16.471 gms. (Clerget and Biot). The cause of these great differences is due partly to variations in the quartz standard and partly to variations in the purity of the light used for illumination.

The old normal weight established for French instruments was 16.35 gms., and this weight is still largely used in technical work with the Soleil-Duboscq saccharimeter. In 1875 the value of Girard and

de Luynes, 16.19 gms., was adopted as the official weight and remained such for more than 20 years, notwithstanding the severest criticism. In 1896 the International Congress of Applied Chemistry at Paris established the value of 16.29 gms. sucrose dissolved at 20° C. in 100 metric c.c., and this is the official weight used at present by the French Ministry of Finance.

**Ventzke or German Sugar Scale.** — The sugar scale most generally used outside of France and the one employed upon all German saccharimeters is that of Ventzke. This scale as originally devised by Ventzke * was based upon the rotation of a solution of pure sucrose of 1.1 sp. gr. $\frac{17.5°}{17.5°}$. It was soon found, however, inconvenient, as well as inaccurate, to make the specific gravity of solution a basis for saccharimetric work, and the grams of sugar in 100 c.c. of solution 1.1 sp. gr. was used for the normal weight; this was determined to be 26.048 gms. weighed in air with brass weights and dissolved at 17.5° C. to 100 metric c.c.

*Mohr Cubic Centimeter Standard.* — With the introduction in 1855 of the Mohr † cubic centimeter (the volume of 1 gm. of water at 17.5° C. weighed in the air with brass weights), the original normal weight of 26.048 gms., designed for metric cubic centimeters, was strangely enough retained and used for determining the 100-degree point of the sugar scale. In this way the standard was established which up to 1900 was the only one recognized for the Ventzke scale, and which at the present time is still the one most commonly used in commercial work. In accordance with this standard, the 100-degree point of the sugar scale is obtained by dissolving 26.048 gms. of chemically pure sucrose (weighed in air with brass weights) in 100 Mohr c.c. at 17.5° C. and polarizing the same in a 200-mm. tube at 17.5° C. in a saccharimeter whose quartz-wedge compensation has also a temperature of 17.5° C. This normal weight calculated to 100 metric c.c. (volume of 100 gms. water at 4° C.) is equal to 26.048 gms. ÷ 1.00234 = 25.9872 gms. (1 Mohr c.c. = 1.00234 metric c.c.).

*Metric Cubic Centimeter Standard.* — On account of the confusion and mistakes resulting from two standards of volume, the International Sugar Commission, at its third meeting in Paris, 1900, advocated the abandonment of the Mohr for the metric cubic centimeter, and in so doing also recommended that the temperature of polarization be made 20° C. The change in temperature from 17.5° C. to 20° C. necessitated a recalculation of the normal weight owing to the difference in specific

* J. prakt. Chem., **25,** 84 (1842) ; **28,** 111 (1843).

† "Chemisch-analytische Titrirmethode" (1886), pp. 44–50.

rotation of cane sugar and quartz at these two temperatures. The calculation is made by the following equation, in which 0.000184 is the coefficient of decrease in specific rotation of sucrose at 20° C., 0.000148 the coefficient of increase in rotation due to the effect of temperature upon wedge and scale, and 0.000008 the coefficient for expansion of the glass observation tube:

$$\frac{26.048}{1.00234}\{1+(0.000184+0.000148-0.000008)\ (20^\circ-17.5^\circ)\}=26.0082$$

gms. The International Commission decided, however, to make the new normal weight exactly 26 gms., and in accordance with its recommendation the following definition for the 100-degree point of the Ventzke sugar scale has been universally adopted: "The 100-degree point of the saccharimeter scale is obtained by polarizing a solution containing 26.000 gms. of pure sucrose (weighed in air with brass weights) in 100 true c.c. at 20° C. in a 200-mm. tube in a saccharimeter whose quartz-wedge compensation must also have a temperature of 20° C." All saccharimeters using the Ventzke scale are standardized at present in accordance with this definition. The difference between the new scale and the old Mohr cubic centimeter scale is very slight, the latter being about 0.03° V. higher, which is too small to be of consequence in ordinary saccharimetric work.

*United States Coast Survey Standard.* — The old original standard of the Ventzke scale was the one adopted by the Department of Weights and Measures of the United States Coast and Geodetic Survey, and was employed for many years by the United States Treasury Department in the Custom House laboratories. The 100-degree point of the scale was taken as the polarization of 26.048 gms. (in vacuo) of pure sucrose dissolved to 100 true c.c. of solution at 17.5° C. and polarized at this temperature in a 200-mm. tube. To avoid the labor of reducing this weight of sugar to vacuo, the flasks employed for the Coast Survey standard were graduated to contain 100.06 true c.c., the excess of 0.06 c.c. being taken to correct the error of weighing the sugar in air against brass weights. These flasks contain 0.174 c.c. less than the old Mohr cubic centimeter flasks (100.234 true c.c.), which difference, unless compensated, would cause the normal weight of 26.048 of pure sucrose to polarize 0.17° V. too high. To save the operators the trouble of making this correction, the correction of 0.17 was applied to the quartz test plates used for controlling the instruments. The computed values of the Coast Survey test plates were thus 0.17° V. lower than the values marked by the instrument makers for the Mohr cubic centimeter standard.

The policy of the Department of Weights and Measures of the United States Coast Survey, in adopting a standard different from that in current use, was unfortunate. It gave rise to much confusion and misunderstanding, and traces of this confusion still exist, notwithstanding the fact that the United States Bureau of Standards, the Custom House, and all other United States Government laboratories have abandoned the old Coast Survey standard and now employ the standard of the International Commission of 26 gms. to 100 true c.c. at 20° C.

According to the work of both Sawyer* and Rolfe,† who have made comparative readings of standard quartz plates upon various saccharimeters, there are many instruments in the United States, even of recent manufacture, which are standardized for a normal weight of 26.048 gms. in 100 true c.c. Whether this condition of affairs is due to a mistaken idea of some manufacturers that the old Coast Survey standard is still recognized officially in the United States, is difficult to say. It is evident, however, that chemists, in order to avoid the considerable errors due to confusion in standards, should state explicitly, in ordering saccharimeters from manufacturers, that their instruments be graduated according to the standard of the International Commission. When purchasing second-hand saccharimeters, chemists should be particularly careful to subject the same to a thorough examination and verification before using.

*Value of the Ventzke in Circular Degrees.* — The rotation value of the 100-degree point of the modern Ventzke scale has been very carefully determined by Schönrock,‡ who found it to equal 34.657 circular degrees for spectral pure sodium light. This is the value used at present by Schmidt and Haensch § in the standardization of all their saccharimeters.

**Bichromate Light Filter.** — Schönrock || has shown that in establishing the 100-degree point of the Ventzke scale by means of sucrose the white light must be filtered through a 1.5-cm. layer of 6 per cent potassium-bichromate solution in order to eliminate the errors of rotation dispersion between cane sugar and quartz produced by the light of shorter wave length at the violet end of the spectrum. This light filter has been adopted by the Physikalisch-Technische Reichsanstalt of Germany and also by the United States Bureau of Standards ¶ in

* J. Am. Chem. Soc. **26,** 990.

† Technology Quarterly **13,** 294. (1905).

‡ Z. Ver. Deut. Zuckerind., **54,** 521.

§ According to statement in a letter to the author.

|| Z. Ver. Deut. Zuckerind., **54,** 521.

¶ Upon its certificates for standardization of quartz plates a sugar degree is thus defined by the United States Bureau of Standards: "A sugar degree is the one-hundredth

defining the 100-degree point of the saccharimeter scale, and its use is imperative for all accurate work. Many saccharimeters have a 3-cm. cell, and for this length of liquid a 3 per cent bichromate solution is sufficient (centimeter length of cell × per cent bichromate = 9). For carbohydrate materials of greater rotation dispersion than cane sugar, such as dextrin, commercial glucose, etc., the author has found it necessary to use a solution of double the above concentration (centimeter length of cell × per cent bichromate = 18) in order to secure constancy of results between different observers for different sources of white light.

In this connection it is important to note that the rotations of the normal weight of sucrose with bichromate-filtered white light and with sodium light, while very closely agreeing, are not absolutely identical owing to the slight differences in optical center of gravity. Measurements by Schönrock* show that, while a normal sugar solution at 20° C. for bichromate filtered white light is exactly equal to the rotation of a quartz plate of 100° V. (34.657 angular degrees), by using sodium light a quartz plate of 100.03° V. (34.667 angular degrees) would be required. The relationship between Ventzke degrees for bichromate filtered white light and monochromatic light of different wave lengths is seen from the following table:†

TABLE XX

*Showing Rotation of Quartz and Sucrose for Different Kinds of Light*

| Source of light. | Mean wave length $\mu\mu$. | Angular rotation, 20° C. | | Degrees Ventzke. |
|---|---|---|---|---|
| | | Quartz plate (1.595 mm.). | Sucrose solution (26 gms. in 100 true cubic centimeters in 200-mm. tube). | |
| White light filtered through 1.5 cm. of bichromate solution, about | 600 | 34.65 | 34.65 | 100.00 |
| Spectral pure sodium light | 589.3 | 34.657 | 34.667 | 100.03 |
| White light, Welsbach, unfiltered, about | 551 | 39.82 | 39.87 | 100.12 |
| Yellow-green mercury | 546.1 | 40.73 | 40.81 | 100.19 |
| Green tantalum | 535 | 42.49 | 42.67 | 100.42 |
| Blue strontium | 460.7 | 58.65 | 59.18 | 100.91 |
| Violet rubidium | 420.2 | 71.78 | 72.87 | 101.52 |

part of the rotation shown by 26 gms. of sucrose dissolved in water and the volume made up to 100 metric cubic centimeters, for light from an incandescent gas mantle passed through 1.5 centimeters of a 6 per cent potassium-bichromate solution, the temperature being 20° C. for graduation, preparation, and observation."

* Z. Ver. Deut. Zuckerind., **54**, 521.

† Compiled from results by Landolt and by Schönrock.

It is seen that while the quartz and sugar exactly agree for bichromate filtered light, the sugar is rotated to a continually greater extent than quartz for light of decreasing wave length. The normal sugar solution, reading 100° V. with filtered white light, was found to read 100.12 degrees with unfiltered white light. The eyes of some observers are more sensitive than those of others to the disturbances of rotation dispersion when unfiltered light is used (owing perhaps to some difference in the pigment of the eye), so that for accuracy and constancy of results in all saccharimetric measurements the bichromate filter should never be omitted.*

**Graduation of Saccharimeter Scales.** — Manufacturers of saccharimeters in establishing the 100-degree point of their sugar scales employ a carefully standardized quartz plate instead of the normal weight of sucrose. The errors and inconveniences incident to the preparation of chemically pure sucrose and to making the solution up to exact volume are thus avoided; the plate, moreover, has the advantage of being a standard which at constant temperature is always unchangeable. Messrs. Schmidt and Haensch† thus describe the method of graduating the scales of their saccharimeters:

"The establishment of the scale divisions of our saccharimeters is made at a *temperature of 20° C.* After fixing the zero point the linear distance of the 100-degree division is determined by means of a normal quartz plate reading exactly 100 degrees and standardized at the Physikalisch-Technische Reichsanstalt. This linear distance is then divided into 100 exactly equal parts, the intermediary divisions being also verified by means of corresponding normal standardized quartz plates. The surfaces of the quartz wedges are made perfectly plane so that a quartz stratum of half thickness corresponds to a half value in the division. Slight errors cannot be prevented, as it is impossible to obtain quartz wedges of the necessary length which are absolutely optically homogeneous throughout. The variableness in the specific rotation of sucrose with concentration of solution is not taken into consideration in the establishment of the scale division, and this must be corrected for by calculation. Aberrations in the scale division caused by impurities in the quartz can be detected by the control observation tube."

The view that the Ventzke scale of modern saccharimeters is corrected for variations in specific rotation of sucrose with concentration,

* Strangely enough, a bichromate light filter is not used upon the saccharimeters of the United States Custom House laboratories, although the United States Bureau of Standards prescribes such a filter in defining the degree of the saccharimeter scale (see note, page 115).

† In a letter to the author.

either by curving the surface of the quartz wedges or by unequal spacing of the scale divisions, is not substantiated by the above statement.

*Effect of Concentration upon Scale Reading.* — A table has been calculated by Schmitz* to correct for the changes in specific rotation of sucrose through varying concentration, which gives the actual sucrose value of each scale division of the saccharimeter. These corrections, which were calculated by Schmitz's formula, $[\alpha]_D = 66.514 - 0.0084153\,c$, would seem in light of more recent work to require considerable modification. The formula of Landolt,

$$[\alpha]_D^{20^\circ} = 66.435 + 0.00870\,c - 0.000235\,c^2, \ (c = 0 \text{ to } 65),$$

calculated from the combined observations of Tollens, and of Nasini and Villavecchia, is regarded as the most accurate at present (see page 176). In the following table the author has recalculated the sucrose values of the Ventzke scale for different concentrations, using Landolt's formula. The values of Schmitz are also given for comparison.

TABLE XXI

*Showing Effect of Concentration of Sucrose upon Saccharimeter Readings*

| Scale division. | Concentration. Grams sucrose, 100 true cubic centimeters, 20° C. | Specific rotation sucrose, 20° C. | Actual sucrose value of scale division. | |
|---|---|---|---|---|
| | | | By Landolt's formula. | By Schmitz's formula. |
| 100.00 | 26.00 | 66.502 | 100.00 | 100.00 |
| 96.00 | 24.96 | 66.506 | 96.00 | 95.98 |
| 95.00 | 24.70 | 66.507 | 94.99 | 94.98 |
| 90.00 | 23.40 | 66.510 | 89.99 | 89.97 |
| 85.00 | 22.10 | 66.513 | 84.99 | 84.96 |
| 80.00 | 20.80 | 66.514 | 79.99 | 79.95 |
| 75.00 | 19.50 | 66.515 | 74.99 | 74.94 |
| 70.00 | 18.20 | 66.516 | 69.99 | 69.93 |
| 65.00 | 16.90 | 66.515 | 64.99 | 64.92 |
| 60.00 | 15.60 | 66.514 | 59.99 | 59.92 |
| 55.00 | 14.30 | 66.511 | 54.99 | 54.92 |
| 51.00 | 13.26 | 66.509 | 50.99 | 50.92 |
| 50.00 | 13.00 | 66.508 | 50.00 | 49.92 |
| 45.00 | 11.70 | 66.505 | 45.00 | 44.92 |
| 40.00 | 10.40 | 66.500 | 40.00 | 39.92 |
| 35.00 | 9.10 | 66.495 | 35.00 | 34.92 |
| 33.00 | 8.58 | 66.492 | 33.00 | 32.93 |
| 32.00 | 8.32 | 66.491 | 32.01 | 31.93 |
| 30.00 | 7.80 | 66.489 | 30.01 | 29.93 |
| 25.00 | 6.50 | 66.481 | 25.01 | 24.94 |
| 20.00 | 5.20 | 66.474 | 20.01 | 19.95 |
| 15.00 | 3.90 | 66.465 | 15.01 | 14.96 |
| 10.00 | 2.60 | 66.456 | 10.01 | 9.97 |
| 6.00 | 1.56 | 66.443 | 6.01 | 5.98 |
| 5.00 | 1.30 | 66.442 | 5.00 | 4.98 |

* Ber., **10**, 1414; Z. Ver. Deut. Zuckerind., **28**, 63, 887.

It will be seen from the preceding table that the greatest deviation of the actual sucrose value from its scale division according to Landolt's equation is only 0.01° V., which is too small to be detected by the ordinary saccharimeter. The maximum error according to Schmitz is 0.08° V.

As regards the concentration of sucrose employed in ordinary saccharimetric work, the variations due to changes in specific rotation may therefore be safely disregarded. The small extent of these variations, which are distributed both above and below the scale division, justifies the policy of the manufacturers in neglecting this factor when establishing the divisions of the saccharimetric scale.

### Verification of Scales of Saccharimeters

On account of the optical imperfections which quartz wedges occasionally possess, it is important that every user of a saccharimeter should verify the accuracy of his instrument.

Owing to the fact that the quartz parts of the saccharimeter are mounted close to the objective of the telescope, the very local imperfections of the wedge system are fortunately unnoticed, since, when the telescope is focused upon the polarizer, the cone of light rays emanating from the different parts of the field covers an area of the compensator equal to the aperture of the analyzer diaphragm (about 6 mm. diameter) and thus distributes and neutralizes any slight local errors due to defects of the quartz. Such defects in the fixed part of the system (small wedge and compensation plate) are of no account, since the rotatory power of this remains constant; the predominant optical defects of the large movable wedge are the only ones which vitiate the results of observation.

Since local optical impurities in the large wedge are diffused over a considerable area, for the reason given above, the errors in the saccharimeter scale never consist of sudden jumps, but only of gradual undulations. It is unnecessary, therefore, as Landolt has shown, to standardize every division of the scale. The errors at every fifth degree, if plotted upon coördinate paper, are sufficient to establish a correction curve from which the error of any division upon the scale can be accurately found (see Fig. 83).

**Verification by Quartz Plates.** — The simplest and easiest method of scale verification, as well as the most accurate, is by means of carefully standardized quartz plates. The cost of a sufficient number of plates to standardize the entire scale is, however, prohibitive, so that the chemist is usually content with a few standard plates for that portion of the scale most used, as 80 to 100 for cane sugar. The pos-

session of a few carefully standardized quartz plates is a necessity for accurate saccharimetric work, not so much for standardization (since the constant error of the scale need be determined but once), but for the determination of zero point, which is necessary with each set of observations.

The standard quartz plates furnished by instrument makers are mounted in metal tubes upon which is stamped the reading that the plates should give upon the particular saccharimeter scale. It is important that this reading be verified by some testing bureau, as slight errors in marking or faults in optical homogeneity of the plate are not uncommon. The surface of the plate when placed in the instrument must be perpendicular to the beams of polarized light which traverse it; for this reason the plates should never be loose in their mountings. On the other hand, the mounting must not press too tightly upon the plate, as optical errors might be produced in the quartz. Rotation of the plate about the axis of its tube should cause no change in the field at the end point. The plate when being used should be brought as close to the analyzer diaphragm as possible in order to give the greatest spread to the cone of light rays emanating from each part of the field. Care must be taken that the standard plate during polarization have exactly the same temperature as that of the quartz wedges of the instrument. If the plate have a temperature above that of the wedges, it will give a reading higher than its true value. The temperature polarization coefficient of quartz is 0.000136, so that the polarization of a plate reading 100° V. at 20° C. would be for 30° C.,

$$100\{1 + (0.000136)(30° - 20°)\} = 100.14° \text{ V.}$$

If plate and instrument are of different temperature, the plate should remain several hours in the trough of the saccharimeter before using, that sufficient time may be given for it to acquire the same temperature. While it is necessary that quartz plate and wedge system have the same temperature, it is not essential that this be the standard temperature for the instrument, since the variations due to temperature are practically the same for plate as for wedge. The slight differences due to effect of temperature upon shape of quartz wedge and upon expansion of nickeline scale are expressed by the formula (Schönrock), $V_{20} = V_t + V_t\, 0.000005(t - 20)$, in which $V_{20}$ and $V_t$ are the readings of the plate at 20° C. and $t$° C. respectively. A standard plate polarizing 100° V. at 20° C. would accordingly polarize 99.99° V. at 40° C. (plates and wedges in each case at same temperature), a variation of 0.01° V. for 20° C. difference, which is negligible in practical work.

**Verification by Pure Sucrose.** — A second means of verifying the saccharimeter scale is with chem cally pure sucrose. The preparation of sucrose of requisite purity is a matter of some difficulty; the method of the International Commission for Unifying Methods of Sugar Analysis * is as follows:

"The purest commercial sugar is purified in the following manner: Prepare a hot saturated aqueous solution, precipitate the sugar with absolute ethyl alcohol, spin the sugar carefully in a small centrifugal machine, and wash in the latter with absolute alcohol. Redissolve the sugar obtained in water, again precipitate the saturated solution with alcohol, and wash as above. Dry the second crop of crystals between blotting paper, and preserve in glass vessels for use. Determine the moisture still contained in the sugar and take this into account when weighing the sugar which is to be used." If a hand centrifugal is not available, the fine crystals of sugar may be filtered and washed free of sirup upon a Buchner funnel. In saturating the sugar solution before precipitation with alcohol, it is well not to heat above 80° C. The sugar solution thus prepared is filtered through a hot-water funnel into the alcohol, stirring vigorously. In this way the sugar is precipitated in the form of fine crystals which are easily dried in the air. Moisture is determined by drying at 105° C.

In the selection of sugar for purification, the finest grades of small domino sugar (polarizing 99.90 to 99.95) have been found in the author's experience to give the best results. Rock-candy crystals, which are sometimes recommended, should never be used; they frequently contain perceptible quantities of acid, with the result that inversion takes place during purification. Complete absence of acidity in sugar and alcohol is necessary.

To verify the 100-degree point of the saccharimeter scale, the normal weight of sugar is weighed into a 100-c.c. flask, dissolved in distilled water, and the solution made up to volume, care being taken that the liquid is well mixed before making up the last few cubic centimeters. The solution, which must be perfectly clear, is then polarized in a 200-mm. tube. The conditions of weight, volume, and temperature required for the saccharimeter must be rigidly observed; the flasks and tubes employed should have been previously calibrated. The average of 10 readings is taken and this result corrected for the moisture in the sugar, the amount of which must be determined in a separate portion with each set of observations. The sugar used for polarization should not be dried in a heated-air or water bath owing to the danger of slight

* Proceedings of Paris Meeting, July 24, 1900.

changes in composition. If the vernier of the scale is set at 0 when the field is matched, the polarization of the sugar corrected for moisture should be exactly 100. In the same manner, other divisions of the saccharimeter scale can be verified by taking fractions of the normal weight (e.g., normal weight × 0.85 = 85-degree point of scale, etc.; see Table XXI).

**Verification by Control Tube.** — The most convenient means of verifying the scale divisions of a saccharimeter when using sucrose is by means of the Schmidt and Haensch control tube.* This method presents the advantage that perfectly pure sucrose does not need to be used; in addition to this, but very few solutions are necessary for verifying the entire scale.

The control observation tube according to Landolt's latest form is shown in Fig. 82. It is telescopic in construction and can be adjusted

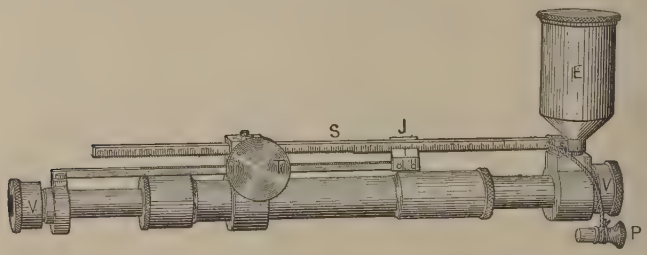



Fig. 82. Control tube for verifying scales of saccharimeters.

so as to give a column of solution for any length between 220 mm. and 420 mm. The length of solution, which is regulated by the screw $T$, is read off upon the scale $S$ by means of the vernier $J$ to 0.1 mm. The tube is surmounted by a funnel $E$, which does not serve for filling, but simply receives the overflow of solution as the tube is shortened. For filling the tube, the funnel is removed and the opening closed by means of a plug ($P$); the tube is then drawn out its full length and filled from the end by unscrewing one of the caps. After rescrewing the cap, the tube is set in an upright position and the funnel replaced as before. After shortening the tube slightly, a few cubic centimeters of solution are poured in the funnel, which is then closed with a small cap to prevent evaporation.

In using the control tube, it is best to begin at the 100-degree point (which is supposed to have been previously verified) of the saccharim-

* Z. Instrument., **4**, 169.

eter scale and work downwards. A sugar solution is first made up of such concentration as to give a reading of 100 degrees at about 400 mm. length of tube. This will be sufficient to test the scale the few divisions above 100 and all divisions below 100 to 55. If the reading, for example, is 100 at 400 mm. upon the tube scale, it should read 105 at 420 mm., 95 at 380 mm., etc. If a deviation be found at any division from the calculated value, other readings should be made at neighboring points of the scale to determine the position of maximum error. After testing the scale to the 55th division (220 mm.), another solution must be prepared which will give a reading of 55 at about 400 mm. and the scale tested down to 30. By proceeding in this way, always making the final point of one series the starting point of the next, the scale can be tested its entire length with 5 solutions. Landolt* has given the following table of concentration for solutions to be used with the control tube in testing the Ventzke scale:

| Number. | Grams of sucrose in 100 c.c. of solution. | Starting point for verification, °V. | Range of scale divisions for verification. |
|---|---|---|---|
| 1 | 12.53 | 100 | 95, 90, 85, .. 60, 55 |
| 2 | 6.89 | 55 | 50, 45, 40, 35, 30 |
| 3 | 3.76 | 30 | 25, 20, 16 |
| 4 | 2.00 | 16 | 15, 10, 9 |
| 5 | 1.13 | 9 | 5 |

In making the readings, the scale of the saccharimeter should first be set at the division which it is desired to verify and then the screw of the observation tube turned until the length of sugar solution gives a matched field. The reading upon the scale of the observation tube is then taken by means of a magnifying glass. The observed length of tube at any division in percentage of the observed length for the 100° V. point gives the actual value of the scale division. To distribute and equalize the errors due to changes in room temperature, warmth imparted to the tube by the hand in making the adjustment, eye fatigue, and other causes, it is well to proceed forward and backward along the tube and not make all the observations for one point at one time. It is desirable to make several sets of readings upon different days and by different observers, and to take the average of the several series. The following results, obtained by the author upon one of the saccharimeters belonging to the New York Sugar Trade Laboratory, will illustrate the method:

* "Das optische Drehungsvermögen" (1898), p. 341.

TABLE XXII

*Verification of S. & H. Saccharimeter, No. 7075*

*Series No. 1*

| Scale division of saccharimeter. | Reading of scale of control tube (average of 10 readings). | Value of scale division (in terms of 100-degree point). |
|---|---|---|
| | mm. | |
| 100 | 396.365 | 100.000 |
| 95 | 376.495 | 94.987 |
| 90 | 356.740 | 90.003 |
| 85 | 336.930 | 85.005 |
| 80 | 316.975 | 79.972 |
| 75 | 297.120 | 74.962 |
| 70 | 277.290 | 69.957 |
| 65 | 257.465 | 64.957 |
| 60 | 237.710 | 59.972 |

*Average of Series*

| Number of series. | Scale division of saccharimeter. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 |
| 1 | 100 | 94.987 | 90.003 | 85.005 | 79.972 | 74.962 | 69.957 | 64.957 | 59.972 |
| 2 | | 95.022 | 90.028 | 85.010 | 80.033 | 75.000 | 69.990 | 64.988 | 59.960 |
| 3 | | 95.008 | 90.005 | 85.005 | 79.985 | 74.998 | 70.003 | 65.012 | 59.980 |
| 4 | | 94.995 | 90.023 | 85.005 | 79.990 | 74.993 | 69.980 | 64.968 | ..... |
| 5 | | 94.985 | 90.015 | 84.985 | 79.985 | 75.003 | 69.995 | 64.997 | 59.990 |
| 6 | | 95.037 | 90.025 | 85.038 | 80.038 | 75.028 | 70.008 | 64.990 | 60.002 |
| Final average | 100.000 | 95.002 | 90.017 | 85.007 | 80.001 | 74.997 | 69.989 | 64.985 | 59.981 |

A similar average made upon another S. & H. saccharimeter (No. 6920) gave

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100.000 | 95.004 | 90.034 | 85.041 | 80.050 | 75.028 | 70.035 | 65.031 | 60.015 |

The results show great exactness of graduation, the error in no instance exceeding 0.05° V.

By marking the degrees of the saccharimeter scale upon a straight line and laying off the observed errors above or below this line for their respective scale divisions, the curve connecting the error points will give the correction for any degree of the scale.

The following diagram (Fig. 83) for the observations of Table XXII will illustrate the method:

To verify the scales of a double-wedge saccharimeter, the scales of both wedges are first set at zero with their verniers for the matched field, any deviation of zero point being corrected by the regulating screw. The working-wedge scale is then verified and its curve of error determined by the control tube in the manner described. The control scale is then compared with the corrected readings of the working scale and its own error curve plotted. A still better direct method is

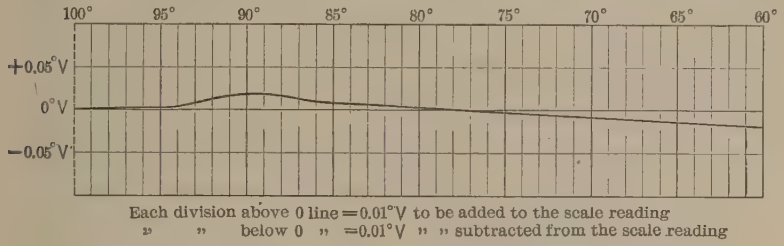



Fig. 83. — Example of diagram for correcting saccharimeter readings.

to set the working wedge at 100 and then verify the control scale from the 0 division upwards by means of the control tube, using the same solutions as for verifying the working scale. If the tube, for example, with a length of 400 mm., gives a reading of 100° V. on the working-wedge scale with control-wedge scale at 0 degrees, then with the working-wedge scale at 100° V. the control-wedge scale should read 5 with a tube length of 380 mm., 10 with a length of 360 mm., etc.

The millimeter scale of the control tube should be verified before the instrument is put to use. The control tube can be employed only upon the large-sized saccharimeters, which have a trough length of 420 mm.

**Verification by Scheibler's* Method of "Hundred Polarization."** — Another means of verifying the scale readings of a saccharimeter is Scheibler's so-called method of "hundred polarization." In this process of verification the polarization of the raw sugar or other product is first determined and then the calculated amount of substance weighed out which should give a polarization of exactly 100. Thus: if a normal weight of 26 grams of a sugar dissolved to 100 c.c. polarizes 82.5 then $\frac{26 \times 100}{82.5} = 31.515$ grams, the weight of sugar dissolved to 100 c.c. necessary to polarize exactly 100. If the polariza-

* Z. Zuckerfabr. Deut. Reiches, **21**, 320.

tion obtained by the calculated weight of sugar is found to be 100, then the original scale reading of the saccharimeter is verified.

### Effect of Temperature upon the Reading of Saccharimeter Scales

In the polarization of sugars and other materials upon quartz-wedge saccharimeters, the effect of temperature upon the scale reading is a most important factor. The saccharimeter is graduated to be used at a fixed temperature (17.5° C. or 20° C.), and in the most carefully regulated sugar laboratories this temperature is maintained throughout the year. But very few laboratories, however, are equipped with the necessary appliances for maintaining a temperature of 20° C. in summer, and the influence of temperature changes upon the saccharimetric readings and the methods for correcting the errors of the same should therefore be considered.

**Temperature Coefficient of Quartz.** — The changes in specific rotation of sugars with variation in temperature are considered on page 178. These changes apply to measurements made upon any kind of polariscope. But with the saccharimeter, as distinguished from the rotating polariscope, there must be considered an additional error due to the influence of temperature upon the quartz compensation of the instrument. This influence has been shown by Schönrock* to be threefold. There is (1) the change in shape of the wedge by expansion or contraction. The coefficient of expansion per 1° C. of quartz perpendicular to its axis ($\eta$) is 0.000013, and parallel to its axis ($\eta'$) is 0.000007. The polarization value of the 100 point of the scale through change in shape of the wedge decreases with increasing temperature by $\eta' - \eta$, or by the coefficient $-0.000006$. There is (2) the change per millimeter thickness in the specific rotation of quartz itself, which for each degree increase in temperature increases by the coefficient 0.000136. The combined temperature coefficient of the wedge system is therefore 0.000130. There is (3) the change due to the expansion and contraction of the material constituting the scale. The error due to this change, together with that resulting from atmospheric humidity, was so great with the old ivory scales that the latter have been replaced in most saccharimeters with the alloy nickeline which has an expansion coefficient per 1° C. of 0.000018. The total correction, therefore, for a quartz-wedge saccharimeter with nickeline scale is 0.000148. The polarization value $w$ for any temperature $t$ is then expressed by the

* Z. Ver. Deut. Zuckerind., 54, 521.

equation $w^t = w^{20}\{1 + 0.000148\,(t - 20)\}$. With saccharimeters whose scale is etched directly upon the wedge itself, as is the case with Schmidt and Haensch instruments of recent construction, the coefficient remains 0.000130.

The above increase in polarization of quartz with increase in temperature necessarily produces a lowering in the readings of the saccharimeter scale, since a smaller thickness of quartz is required for compensation. With sugars which undergo a decrease in specific rotation with increase in temperature, the combined influences are in one direction and the error thus introduced may be considerable. With sucrose, for example, the temperature coefficient of polarization becomes at 10° C. 0.000390 (0.000148 + 0.000242), at 20° C. 0.000332 (0.000148 + 0.000184), and at 30° C. 0.000269 (0.000148 + 0.000121).

**Temperature Coefficient of Sucrose.** — The variation in the Ventzke reading of the normal weight of pure sucrose for 1° C. change in temperature has been found by different authorities to be as follows:

| | |
|---|---|
| Andrews* | 0.0300 |
| The United States Coast and Geodetic Survey | 0.0293 |
| Wiley† | 0.0314 |
| Prinsen Geerligs‡ | 0.0300 |
| Watts & Tempany§ | 0.0310 |
| Average = | 0.0303 |

The average temperature coefficient of the above is therefore 0.000303, which agrees with the figure of Schönrock for 25° C. (0.000148 + 0.000152) = 0.000300. For temperatures between 20° and 30° C. the general equation $V^{20} = V^t\{1 + 0.0003\,(t - 20)\}$ may be used for changing the Ventzke reading ($V^t$) of pure sucrose at any temperature $t$ to the reading ($V^{20}$) at 20° C.

**Temperature Coefficients of Other Sugars.** — The temperature coefficients of other common sugars for readings upon the Ventzke scale are given in the following table. The temperature coefficient for fructose and invert sugar are for readings made upon the negative scale of the saccharimeter; while the coefficients of these sugars decrease the same as those of the dextrorotatory sugars, the direction of the decrease in both cases is towards the 0 point and therefore opposite to each other (as indicated by the arrow points).

* Technology Quarterly, Mass. Inst. Technology, May (1889), 367.
† J. Am. Chem. Soc., **21**, 568.
‡ Archief Java Suikerind, July (1903).
§ West Indian Bull., Vol. III, p. 140.

TABLE XXIII

*Giving Temperature Coefficients of Different Sugars for Ventzke Scale*

| Sugar. | *A* $[\alpha]_D^{20°}$. | *B* Change in $[\alpha]_D^{20°}$ for 1° C. increase. | *C* Temperature coefficient $\frac{B}{A}$. | Temperature coefficient of reading upon Ventzke scale for 1° C. increase. $C$ + coefficient for quartz (−0.000148). | |
|---|---|---|---|---|---|
| Fructose..... | −92.50 | +0.625 | −0.006757 | −0.006905 | →0 |
| Invert sugar... | −20.00 | +0.312 | −0.015600 | −0.015748 | →0 |
| Lactose..... | +52.53 | −0.070 | −0.001332 | −0.001480 | 0← |
| Maltose..... | +138.04 | −0.095 | −0.000688 | −0.000836 | 0← |
| Glucose..... | +53.23 | No change | No change | −0.000148 | 0← |

In case a mixture of sugar is polarized upon a saccharimeter, the combined influence of the temperature coefficients of each sugar must be considered. To arrive at a better understanding of the use of such coefficients the following special problem is considered:

It is desired to find the amount of fructose and of invert sugar which, mixed with 26 gms. of pure sucrose, will give a constant saccharimeter reading at all temperatures.

It has been shown that 26 gms. of pure sucrose, reading 100° V. at 20° C., undergo a decrease of 0.03° V. with 1° C. increase in temperature. Since a fructose solution reading −1° V. undergoes a decrease in polarization of 0.0069° V. (Table XXIII), then $\frac{0.03}{0.0069} = -4.35°$ V., the scale reading of the required amount of fructose. Since 0.1869 gm. of fructose in 100 metric c.c. reads −1° V. at 20° C. in a 200-mm. tube, then $4.35 \times 0.1869 = 0.813$ gm., the required amount of fructose. 26 gms. sucrose and 0.813 gm. fructose (3.13 per cent of the weight of sucrose) will give, therefore, a constant saccharimeter reading at all temperatures.

In the same way for invert sugar, $\frac{0.03}{0.01575} = -1.90°$ V., the scale reading of the required amount of invert sugar. Since 0.8645 gm. invert sugar in 100 metric c.c. reads −1° V. at 20° C. in a 200-mm. tube, then $1.90 \times 0.8645 = 1.642$ gms., the required amount of invert sugar. 26 gms. sucrose and 1.642 gms. invert sugar (6.32 per cent of the weight of sucrose) will give, therefore, a constant saccharimeter reading at all temperatures.

The effect of 1° C. increase in temperature upon the reading of 1 per cent each of sucrose, fructose, and invert sugar for a normal weight of 26 gms. in 100 metric c.c. is given in the following table:

TABLE XXIV

*Showing Influence of Temperature upon Ventzke Reading of* 1 *per cent Sucrose, Fructose, and Invert Sugar for a Normal Weight of* 26 *gms. Solutions made up to Volume at Temperature of Polarization*

1 per cent sucrose $= \frac{0.03}{100} = -0.0003°$ V. for 1° C. increase.

1 per cent fructose $= \frac{0.03}{3.13} = +0.0096°$ V. for 1° C. increase.

1 per cent invert sugar $= \frac{0.03}{6.32} = +0.0048°$ V. for 1° C. increase.

(− denotes change toward the left. + denotes change toward the right.)

Since the influence of temperature upon the rotation of glucose is so small as to be negligible, the change in rotation for 1 per cent invert sugar should be the same as that for 0.5 per cent fructose, or +0.0048° V. This is the result actually obtained, so that the calculation is verified.

## SHALL SACCHARIMETERS BE ADJUSTED TO VARIABLE TEMPERATURES?

The International Commission* has provided that "for laboratories in which temperatures are usually higher than 20° C., it is permissible to graduate saccharimeters at any suitable temperature, providing that the volume be completed and the polarization made at the same temperature." The Commission has neglected, however, to say how this graduation shall be made. It is evident that in order to have a normal weight of sucrose, under the conditions prescribed for a saccharimeter at 20° C., polarize 100 at 25° C. or 30° C., the compensating thickness of quartz in the wedge system must be made thinner for each part of the scale in order to counterbalance the decrease in specific rotation of sucrose.

Owing, however, to the confusion and mistakes which would arise in the use of standard plates with saccharimeters of different compensating power, a better plan would be to make no change in the instrument itself, but to alter the conditions of polarization, such, for example, as increasing the normal weight of sugar, or increasing the length of the observation tube, or decreasing the volume of the flask, any one of which means will bring the polarization of pure sucrose to 100 for any desired temperature above the standard. Since odd lengths of tube or volume of flask are undesirable as well as confusing, a change in the normal

* Proceedings of Paris Meeting, July 24, 1900.

weight of sucrose is the simplest of all means of correction. The method of calculation can be understood from the following example.*

What would be the normal weight at 25° C. for a quartz-wedge saccharimeter standardized at 20° C. for 26 gms. sucrose dissolved to 100 true c.c. and polarized in a 200-mm. tube?

The temperature coefficient of the specific rotation of sucrose at 22.5° C. is − 0.000168 (Schönrock). The temperature coefficient of the nickeline scale and quartz wedge is 0.000148; the expansion coefficient for the glass observation tube is 0.000008. The new normal weight would then be

$$26{,}000\ \{1+(0.000148+0.000168-0.000008)\ (25-20)\} = 26.040 \text{ gms.}$$

dissolved to 100 true c.c. in a flask standardized at 25° C.

When saccharimeters are employed constantly in the investigation of pure sucrose solutions, it might be advisable to make a change such as the above in the normal weight. But for varied work with different classes and mixtures of sugars whose specific rotations are affected in opposite ways by changes in temperature, it is inaccurate to make alterations based upon the change in properties of one single sugar. The results obtained upon saccharimeters differently standardized are then no longer comparable. The sucrose normal weight is frequently employed upon mixtures of sucrose with other sugars; in such cases changes in normal weight to correct for rotatory changes in the sucrose alone are wholly unwarranted. In view of the fact that the work of saccharimeters is usually of a varied character, it seems best to leave the scale and standard conditions of the instrument unchanged. The chemist should work wherever possible under the conditions of temperature prescribed for his saccharimeter, and when this cannot be done he should correct his readings as well as possible by a factor established for the particular product which is being examined.

It must always be borne in mind that while the saccharimeter scale is established for the rotation of sucrose, its divisions indicate percentages only when pure sucrose is being polarized; in all other cases the scale division becomes a mere conventional number (degrees Ventzke, degrees polarization, degrees sugar scale, etc.) which the analyst must interpret according to his particular needs.

* This example is from a calculation supplied by the Physikalisch-Technische Reichsanstalt, in reply to a suggestion by the author to use the old Mohr c.c. normal weight 26.048 gms. (17.5° C.) for true c.c. at 25° C. The old normal weight would give a reading of 100.031° V. when dissolved in 100 true c.c. in a flask standardized at 25° C. If the true c.c. flask standardized at 20° C. be used at 25° C., this error would be reduced to 100.019° V., which is within the limits of error for observation.

## Description of Saccharimeters

### *Tint Saccharimeters*

The saccharimeter of Soleil as modified by Ventzke and Scheibler in Germany and by Duboscq in France consists of an adaptation of the quartz-wedge compensation to the polariscope of Robiquet (p. 86).

**The Soleil-Ventzke-Scheibler Saccharimeter.** — The construction and arrangement of the optical parts in the Soleil saccharimeter as modified by Ventzke and Scheibler are shown in Fig. 84. *A* is a Nicol prism and *B* a plate of left or right rotating quartz cut perpendicular to its optical axis; these constitute the tint producer and are mounted

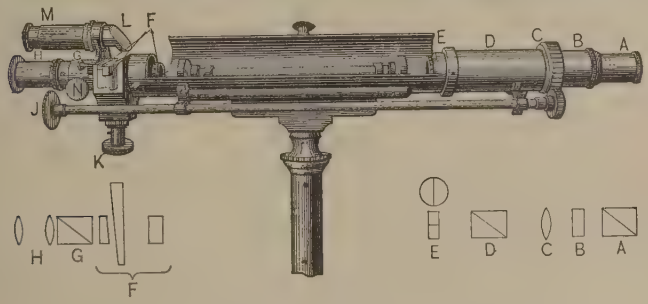



Fig. 84. — Soleil-Ventzke-Scheibler tint saccharimeter.

in a movable sleeve which can be rotated by a rod and pinion from *J*. *C* is a condensing lens, *D* the polarizer, and *E* a Soleil double quartz plate (p. 86). The quartz compensation is at *F*, the analyzer at *G*, and telescope at *H*. In using the instrument the telescope is focused upon the bi-quartz plate, so that the dividing line is sharply defined. The zero point of the scale is then determined by turning *K* until both sides of the field have the same tint (in the manner described on p. 88). By rotating the regulator or tint producer from *J*, the tint which is most sensitive to the eye of the observer is obtained. This tint, which is different for different eyes, is usually of a very delicate violet or pearl color; it will of course vary according to the angle with which the Nicol *A* is set with reference to the Nicol *D* of the polarizer. In order to remove the disturbances in transition tint due to colored solutions (which cannot be remedied in the Robiquet polariscope), the adjustment of the regulator is changed until the tint is again of greatest sensitiveness. With very dark solutions the transition tint is almost a shadow owing to the absorption of color.

**The Soleil-Duboscq Saccharimeter.** — The Soleil saccharimeter as modified by Duboscq, the type of tint instrument used in France, differs from the form previously described in that the Nicol producing the sensitive tint is situated in the eyepiece of the telescope, as shown by *N* in Fig. 85. The latter is rotated by a milled ring *B* until the sensitive tint is produced with the quartz plate *C*, which in the Duboscq instrument is situated between the analyzer and the objective of the telescope. The telescope is focused upon the Soleil double plate at *R*

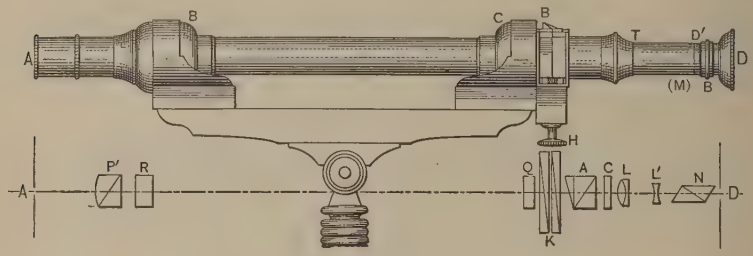



Fig. 85. — Soleil-Duboscq tint saccharimeter.

by moving the eyepiece *D* in or out; longitudinal guides prevent any lateral rotation which might disturb the tint. In the Duboscq instrument the two wedges of the compensator are of equal size, and, being driven past each other by the pinion in opposite directions, give a stratum of quartz of variable thickness. A scale and vernier, which follow the wedges in their movement, indicate the reading.

According to Landolt,* the average error of adjustment with the Soleil saccharimeter is ± 0.2 degree of the scale. The instrument has the same objection as the Robiquet polarimeter, in being unsuited to the color-blind. The adjustment of end point to color is also much more fatiguing to the eye than adjustment to uniformity of shade. Owing to these objections the color saccharimeter, although 20 years ago the standard instrument, is but little used at the present time. Its use is in fact condemned by the Imperial Testing Bureau of Germany.

### *Half-shadow Saccharimeters*

The various types of half-shadow saccharimeter used at the present time consist simply of an adjustment of the quartz-wedge compensation to some one of the half-shade polarizers previously described. The principal forms are the double-field saccharimeter with Jellet-Cornu

* "Das optische Drehungsvermögen" (1898), p. 348.

polarizer; the double-, triple-, and concentric-field saccharimeters with Laurent plate; and the double- and triple-field instruments with Lippich polarizer.

**Saccharimeter with Jellet-Cornu Prism.** — A single-wedge half-shadow saccharimeter with Jellet-Cornu prism as polarizer is shown in Fig. 86.

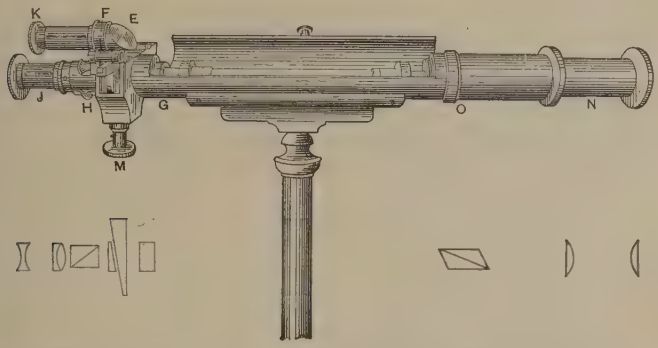



Fig. 86. — Single-wedge saccharimeter with Jellet-Cornu prism.

*N*. Sliding sleeve containing condensing lens.
*O*. Modified Jellet-Cornu prism (Schmidt and Haensch prism).
*E*, *F*. Parts of quartz-wedge compensation.
*H*. Analyzer.
*J*. Telescope, which is focused upon the dividing line of the split prism at *O*.
*K*. Microscope for reading scale.

The above saccharimeter, which 15 years ago was the standard form of instrument employing the Ventzke scale, is at present almost entirely replaced with saccharimeters using the Lippich polarizer.

**Laurent's Saccharimeter.** — As a type of the saccharimeters constructed by French instrument makers, the Laurent instrument shown in Fig. 87 is described. The arrangement of polarizer, half-wave plate, and device for regulating the half-shadow angle is identical with that of the Laurent polarimeter (Fig. 72). The divided circle and rotating analyzer of the latter, however, are replaced in the saccharimeter by the quartz-wedge compensation.

The saccharimeter is adjusted to its zero point by first turning *G* until the two halves of the field agree in shade. If it should be found that one side of the field has more of a reddish tinge than the other at the end point, the screw *F*, which controls the analyzer, is turned so as to darken slightly the side of the field most colored. The screw *G* is

then turned again to equality of shade; if there is still a difference in color, $F$ is moved slightly as before, and $G$ again turned to equality of shade. By proceeding cautiously in this way the observer will at length reach the point where both sides of the field correspond in shade and color. When this point is reached the screw $T$ is turned until the 0 of

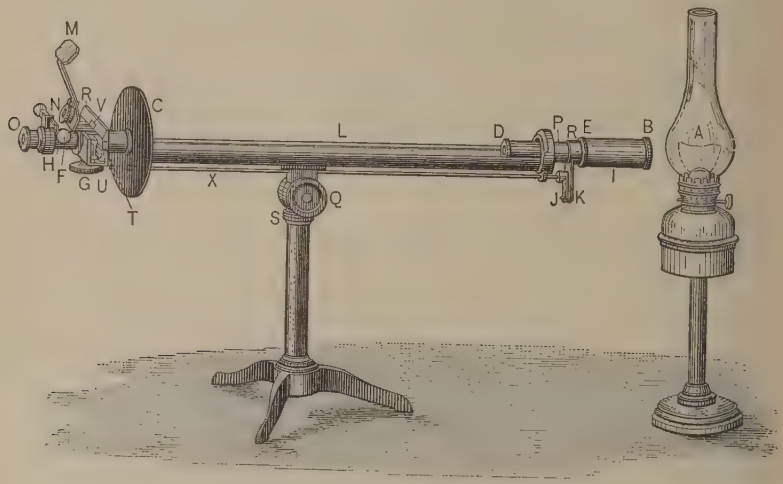



Fig. 87. — Laurent's single-wedge saccharimeter.

*A*. Lamp for producing white light (oil, gas, electricity, etc.), placed 200 mm. from *B*.
*B*, *E*, *R*, *K*, *J*, *X*, *U*, *D*, *L*, the same as under Laurent polarimeter (Fig. 72).
*R*. Saccharimeter scale, which with vernier *V* is illuminated by light reflected from *A* by the mirror *M*.
*N*. Magnifying glass for reading scale and vernier.
*G*. Screw for moving quartz wedges of the Soleil compensator.

the scale coincides with the 0 of the vernier. This adjustment should be verified by taking a number of check readings.

The 100-degree point of the Laurent saccharimeter scale corresponds to a rotation of 21° 40′, the value given by French physicists to the rotation of the 1-mm. plate of quartz. The normal weight for this angular displacement, as previously noted, is 16.29 gms. sucrose for 100 true c.c. polarized in the 200-mm. tube. The Laurent saccharimeter is also manufactured with a scale adapted to the so-called International normal weight of 20 gms. The instrument is provided with double or triple field, as desired. The scale divisions extend from 0 to 110 to the right.

*"Plaque Type."* — The 100-degree point of the Laurent saccharimeter is verified by a standard plate of quartz 1 mm. thick. This standard plate "plaque type" also serves for the polarization of levorotatory solutions. With the plate in the trough of the instrument, the zero point of the scale is transferred to 100; levorotatory solutions are then simply read backwards upon the scale, the reading being the difference between readings of plate and solution. A solution, for example, reading 67.4 with the 100-degree plate in position has a polarization of −32.6. This method of polarizing levorotatory solutions is of course applicable to all single-wedge saccharimeters.

A 100-degree Laurent "plaque type" was remounted by the author and sent to the United States Bureau of Standards for a certification as to its angular rotation and its value in sugar degrees upon a saccharimeter employing the Ventzke scale. The rotation of the plate for sodium light of 589.23 $\mu\mu$ wave length was given as $+21.713 + 0.003\ (T - 20) \pm 0.004$, and the rotation in sugar degrees as +62.65. The same plate read by the author upon a late-model Schmidt and Haensch saccharimeter gave a reading of +62.64, and upon a late-model Frič saccharimeter (Bates modification) a reading of +62.65. These readings of the "plaque type" not only prove the perfect identity of the Ventzke sugar scales employed by two different manufacturers, but also permit the establishment of the exact ratio between the French and German normal weights; for all other conditions as to the temperature and volume are the same in both these countries. The ratio 100 : 26 gms. :: 62.65 : $X$ shows that the ratio of the German normal weight to the French normal weight is as 26 gms. to 16.289 gms., or, in even hundredths, 16.29 gms., which is identical with the official normal weight prescribed in France.

**Duboscq-Pellin Saccharimeter.** — The Duboscq-Pellin saccharimeter for white light, as regards position of polarizer, half-wave plate, quartz-wedge compensation, etc., is the same as that of the Laurent. The concentric field of the Pellin saccharimeter requires a somewhat different cutting of the half-wave plate, but in other respects the two saccharimeters are very much alike.

The saccharimeter with Lippich polarizer is the form most generally preferred at present. The half-shadow angle between the prisms of the polarizer is usually between 5 and 8 degrees; it can be measured approximately by noting the interval between the points of maximum light extinction each side of the zero point. The degrees Ventzke between the two points of maximum darkness multiplied by 0.34657 gives the angle of the half shadow.

**Schmidt and Haensch Saccharimeters.** — A single-wedge Schmidt and Haensch saccharimeter upon tripod support with electric attachment for illumination is shown in Fig. 88.

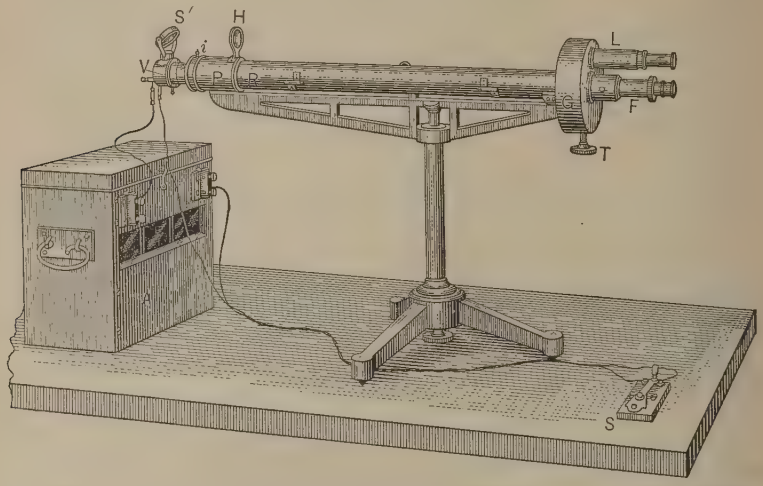



Fig. 88. — Single-wedge Schmidt and Haensch saccharimeter with electric attachment for illumination.

*V*. Detachable end containing lamp and for inserting cell of bichromate solution.
*P*. Position of Lippich polarizer for double or triple field.
*G*. Casing of sheet brass for protecting wedges from dust.

The method of scale illumination in Schmidt and Haensch saccharimeters is shown in Fig. 89 which gives the arrangements of parts for a double-wedge instrument. The light from the lamp is focused upon the small window $a$ in the wedge housing, and is reflected from the mirror $b$ through the ground-glass plate $c$ upon the scale from which it is reflected through the prism $p$ into the microscope whose objective is at $d$ and eyepiece at $f - g$. The working wedge is operated by the screw $A$ and the control wedge by the screw $K$. The appearance of the scale of this instrument as viewed through the microscope is shown in Fig. 80.

The latest and most improved type of Schmidt and Haensch saccharimeter is the double-wedge apparatus shown in Fig. 90. The instrument is mounted upon a bock or trestle support, and for saccharimeters which are in constant use this method of mounting is most satisfactory as it insures perfect rigidity and accurate alignment. The wedges are moved by milled screw heads at $A$ and $K$ which are so

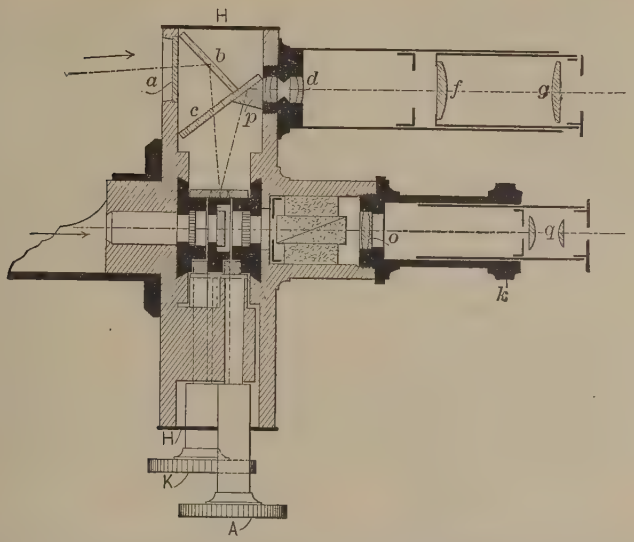



Fig. 89. — Device for illuminating scale of Schmidt and Haensch saccharimeter.

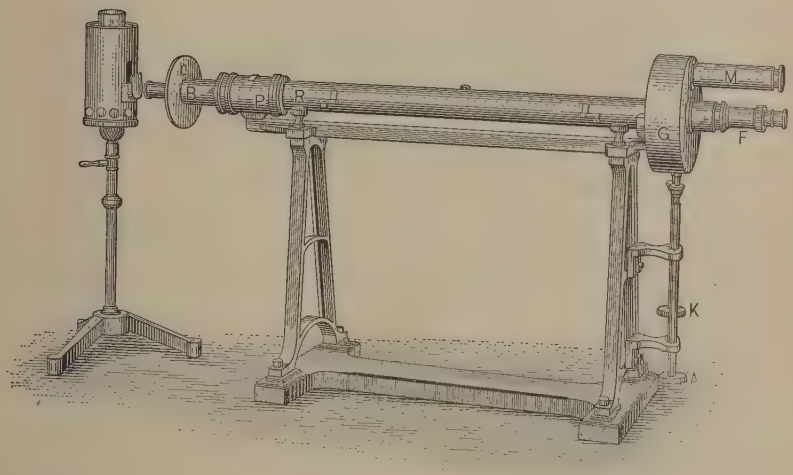



Fig. 90. — Double-wedge Schmidt and Haensch saccharimeter upon bock support.

placed that the hand can rest upon the table during adjustment. The screw $K$ moving the control wedge can be fastened with a clamp, and is placed at a slightly higher elevation to prevent liability of confusion.

**Peters's Saccharimeter.** — Very similar in construction to the above apparatus is the saccharimeter of Peters shown in Fig. 91. The long tube $R$ prevents placing the light too close to the polarizer. The bichromate cell is placed within $S$; the cover $C$ of the trough is not hinged but simply slides over or under the tube. The scale in the

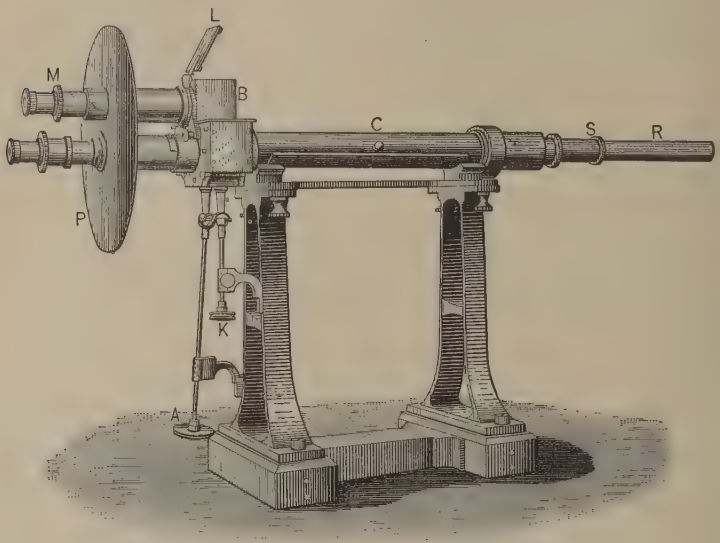



Fig. 91. — Double-wedge Peters saccharimeter.

sheet-metal housing is illuminated by light reflected from the mirror $L$; a black paper disc $P$ protects the eye against the glare of the observation lamp.

**Frič's Saccharimeter.** — The half-shadow saccharimeters of J. and J. Frič are very similar in construction to the instruments previously described except in the method of scale illumination. In the latest types of Frič saccharimeter a part of the light, as it passes from the source of illumination through the diaphragm at the end of the instrument, is reflected through a system of mirrors and lenses upon the scales. This illuminating attachment is shown in the Bates saccharimeter ($L$ in Fig. 94), but the distinctive feature of the Frič illumi-

nating device is at the scale end of the instrument as shown in Fig. 92. The light from $L$ is reflected from the mirror $A$ (which in the instruments with enclosed wedges is stationary) through the milk-glass plate $B$ upon the scale $C$, the latter in the latest Frič saccharimeters being made of glass. The light from $C$ is reflected from the mirror $D$ through the focusing lens $E$ to the eye of the observer. The divisions of the scale illuminated in this manner appear with great distinctness. The Frič double-wedge instruments are provided with separate focusing lenses for reading the working and control scales. The lens mountings and the milk-glass plates for the two wedge systems are usually of different colors in order to prevent confusion.

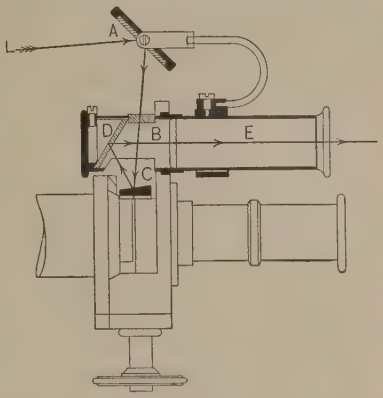



Fig. 92. — Device for illuminating scale of Frič's saccharimeter.

### Saccharimeters with Variable Sensibility

Of the instruments previously described, the French saccharimeters, using a Laurent half-wave plate and employing monochromatic or bichromate-filtered white light, are the only forms of apparatus which permit a variation of the half-shadow angle to suit the requirements of greatest sensibility.

In all the Schmidt and Haensch saccharimeters the half-shadow angle is fixed. An attachment for shifting the large prism of the Lippich polarizer and regulating the half-shadow angle has been supplied by some manufacturers. While this regulating device presents certain advantages, it has been condemned by Landolt * on the ground that every change in the half shadow introduces a change in the zero point which has to be corrected by rotating the analyzer until the field is again evenly illuminated at the zero point — an impossible remedy in a saccharimeter with fixed analyzer.

**Bates's Saccharimeter.** — To obviate the objection last named, Bates† has devised an attachment which rotates the analyzer automatically and makes it possible to correct the zero-point error for any change

* "Das optische Drehungsvermögen," 351.

† U. S. Bur. Stand. Bull., Vol. 4, p. 461; Z. Ver. Deut. Zuckerind., **58**, 105.

in the half-shadow angle without resetting the scale. The principle of the Bates saccharimeter can be understood from Fig. 93.

Let $OP$ be the direction of the plane of the large Nicol and $ON$ that of the small Nicol in a Lippich polarizer, let $AZ$ be the plane of the analyzer at right angles to $OB$ the bisection of the half-shadow angle $PON$ or $\alpha$. We will suppose for a moment that the intensities of light in $OP$ and $ON$ are equal and that the plane of the large Nicol be moved from $OP$ to $OP'$ forming with the plane of the small Nicol the new

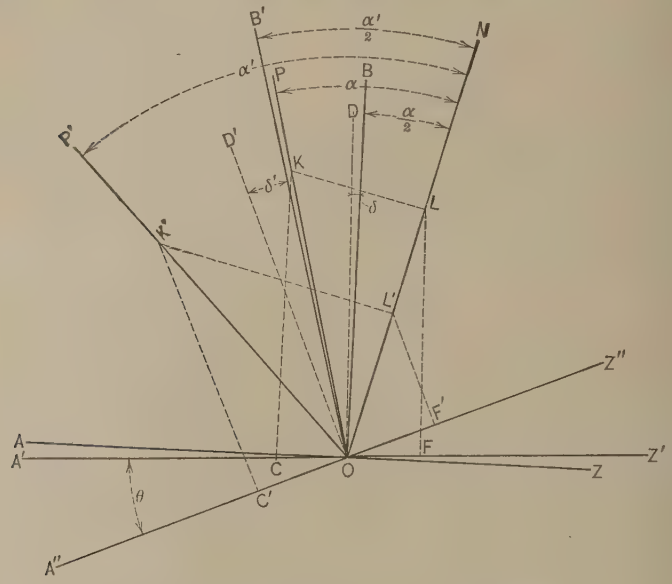



Fig. 93. — Illustrating principle of Bates's saccharimeter.

angle $P'ON$ or $\alpha'$. To obtain uniformity of field at the zero point for the new angle $\alpha'$ the bisection $OB$ must be moved to $OB'$. It will be seen from the diagram that the angle $BOB' = \frac{\alpha'}{2} - \frac{\alpha}{2} = \frac{\alpha' - \alpha}{2} = \frac{POP'}{2}$. To correct, therefore, for the displacement of zero point, assuming the intensities of light to be always the same for both Nicols, the plane of the analyzer must be moved through one half the angular displacement of the large Nicol of the polarizer.

In the Lippich system, however, the intensities of light are not equal for the large and small prisms of the polarizer. A part of the light is

extinguished in the small Nicol and there is also a loss from reflection and absorption. We will consider first the light lost by absorption.

Let $OK$ = amplitude of light from large Nicol. Draw $KL \perp ON$; then $OL$ = amplitude of light from small Nicol; the plane of the analyzer $AZ$ must then be moved to $A'Z'$ that the amplitudes $OC$ and $OF$ be equal in each half of the field. The angles $AOA'$ and $BOD$, through which the plane of the analyzer and its perpendicular have moved, is $\delta$ or the change from the true zero point when the intensities of light in $OP$ and $ON$ are equal, in which case $\alpha = 0$.

We will suppose in order to increase the intensity of light for the half shadow that the plane $OP$ of the large Nicol be moved to $OP'$ increasing $\alpha$ to $\alpha'$. The amplitude $OK'$ remains the same as $OK$. Draw $K'L' \perp ON$; then the amplitude in $ON = OL'$. The plane of the analyzer must now be moved to $A''Z''$ in order that the $\perp$s $K'C'$ and $L'F'$ cut off the equal amplitudes $OC'$ and $OF'$ in the two halves of the field. $OD'$ which is $\perp A''Z''$ will then form with $OB'$, the bisection of $\alpha'$, the new angle $\delta'$. The angle $\theta = DOD'$ through which the analyzer has moved from its previous position is expressed by the equation

$$\theta = \delta' + \frac{\alpha' - \alpha}{2} - \delta.$$

In the polariscope of Bates (Fig. 94) the analyzing Nicol and the large Nicol of the polarizing system are mounted in bearings and are joined by gears with a connecting rod. The milled head, which operates the driving mechanism, is shown at $H$. When the milled head is turned the two Nicols are rotated and the design of the gears is such that the analyzing Nicol always receives one half the angular displacement of the large Nicol of the polarizing system. Above the milled head is a circular scale which shows the polarizing angle for any position of the Nicols. In moving the plane of the large polarizing Nicol through the angle $POP'$ (Fig. 93) the rotating device of Bates's polariscope moves the plane of the analyzer through the angle $BOB'$. In this way the zero-point error of the instrument will always be equal to the value of $\delta$ for any angle of the half shadow, assuming that the zero had been previously adjusted for $\alpha = 0$. If the zero point of the instrument be set for any value of the half shadow $\alpha$, and $\alpha$ be then changed to $\alpha'$, the zero will have an error of $\delta' - \delta$ $\left(\text{the analyzer having rotated } \frac{\alpha' - \alpha}{2}\right.$, this value disappears from the equation

$$\left. \theta = \delta' + \frac{\alpha' - \alpha}{2} - \delta \right).$$

The calculated values of $\delta$ in Ventzke degrees for different values of the half-shadow angle $\alpha$ according to the two equations,

$$\tan \delta = \tan^3 \frac{\alpha}{2} \quad \text{and} \quad \tan \delta = \frac{1 - \cos \alpha \sqrt{0.92}}{1 + \cos \alpha \sqrt{0.92}} \tan \frac{\alpha}{2}$$

(see p. 96), are given in the following table.

TABLE XXV

*Giving Calculated Values of Error in Zero Point for Bates's Saccharimeter*

VALUES OF $\delta$ IN VENTZKE DEGREES.

| Values of $\alpha$ circular degrees. | I By formula $\tan \delta = \tan^3 \frac{\alpha}{2}$. | II By formula $\tan \delta = \frac{1 - \cos \alpha \sqrt{0.92}}{1 + \cos \alpha \sqrt{0.92}} \tan \frac{\alpha}{2}$. |
|---|---|---|
| 1° | 0.003 | 0.033 |
| 2 | 0.004 | 0.064 |
| 3 | 0.005 | 0.096 |
| 4 | 0.008 | 0.129 |
| 5 | 0.014 | 0.164 |
| 6 | 0.024 | 0.205 |
| 7 | 0.038 | 0.249 |
| 8 | 0.057 | 0.299 |
| 9 | 0.080 | 0.352 |
| 10 | 0.110 | 0.412 |
| 11 | 0.150 | 0.482 |
| 12 | 0.192 | 0.554 |

The values of $\delta$ in the second column are greater than those in the first column by $0.03\,\alpha$. The true values of $\delta$ according to Bates lie between those calculated by the two equations and will vary according to the construction of the instrument. This true value of $\delta$ will be the value by the first formula $\pm c\,\alpha$ in which $c$ is a constant for each individual Lippich system. If a Bates saccharimeter be set, therefore, for $\alpha = 0$, the calculated change in zero point for variations in $\alpha$ can be easily applied to the scale reading. If the instrument be set for any particular value of $\alpha$, as 8 degrees, the half-shadow angle may be increased or diminished several degrees from this point without introducing a change in zero greater than $0.1°$ *V*.

The Bates saccharimeter, constructed by Josef and Jan Frič of Prague, is at present the standard instrument of the United States Customs Service. While the apparatus presents several advantages over the ordinary saccharimeter, the mechanical difficulties of construction make it expensive. In its present commercial form the instrument is not provided with a bichromate light filter. While this

omission may occasion no serious error in the polarization of colored solutions (as of low-grade sugar-house products), a bichromate light filter is required in the examination of high-grade cane sugars, starch-conversion products, and many other substances. An absorption cell for this purpose should be placed just in front of the aperture between the saccharimeter and the source of light. A very commendable feature of the Bates instrument is the thermometer (*T* Fig. 94) which indicates the temperature of the quartz wedges.

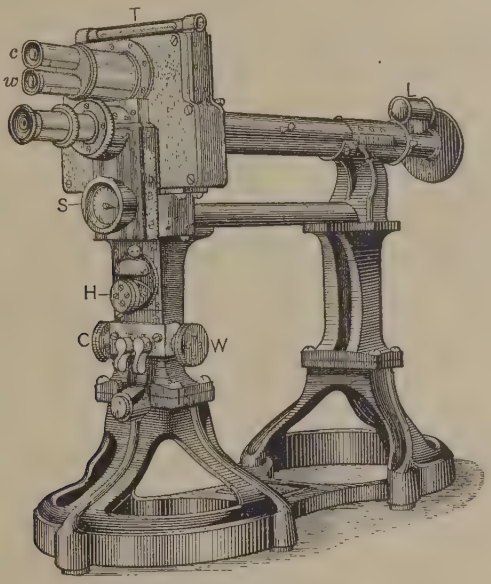



Fig. 94. — Bates saccharimeter with variable sensibility.

*W*, milled head for operating working wedge.
*C*, milled head for operating control wedge.
*w*, microscope for reading working wedge scale.
*c*, microscope for reading control wedge scale.
*S*, scale indicating "degrees of brightness" or half-shadow angle.

### Saccharimeters with Magnified Scale.

For special kinds of work involving the investigation of products with a narrow range in composition, saccharimeters have been constructed with a limited magnified scale. The saccharimeter devised by Stammer,* shown in Fig. 95, for polarization of sugar beets is an

* Z. Ver. Deut. Zuckerind., **37,** 474.

example of such an instrument. In this apparatus a magnified scale, reading from 0 to 35, is attached to the side of the instrument at the observer's left and permits the reading of polarizations with the unaided eye. The pointer of the scale $P$ is moved by the tension roller $R$, which is connected by a small steel chain with the movable quartz wedge.

To adjust the saccharimeter the field is brought to a uniform shade by turning $K$ when the 0 of the wedge scale and vernier at $Q$ should coincide. If the latter is not the case, coincidence is affected by turn-

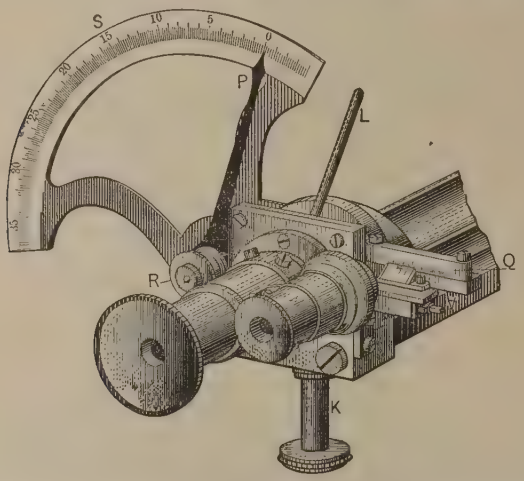



Fig. 95. — Stammer's saccharimeter with magnified scale for polarizing sugar beets.

ing the regulating key $V$. In this position the pointer $P$ should mark exactly the zero division of the large scale $S$. Should there be any deviation the error is corrected by turning the adjusting lever $L$ until the pointer is exactly at 0. Turning the screw $K$ to any division upon the wedge scale $Q$ should then give the same reading upon the scale $S$. If this is not the case the error is corrected by turning a small control screw upon $R$ which increases or diminishes the diameter of the roller. The adjustment is one which requires considerable care and should be checked repeatedly.

Saccharimeters of the above type are especially adapted for the polarization of mother beets for seed production; they are constructed for tubes of 200 mm., 400 mm., and 600 mm. length.

Similar to the above instruments for sugar-beet analysis, saccharim-

eters have been constructed with a magnified scale reading between 80 and 100 for polarization of sugars. These are manufactured usually only for use with tubes 400 mm. long, and employ a normal weight of 26 gms. to 100 c.c. solution. Doubling the length of observation tube necessitates of course doubling the interval between the scale divisions and thus facilitates the reading.

Instruments with a magnified limited scale will be found to relieve eye fatigue, where large numbers of analyses of a single product have to be performed. With one person to prepare the tubes of sugar solutions, a second to manipulate the saccharimeter, and a third to note the readings, a large number of polarizations can be made in a very short period of time.

### Conversion Factors for Polariscope and Saccharimeter Scales

In the following table factors are given for converting 1 degree of the various polariscope scales into its equivalent in circular degrees, or in degrees of the different saccharimetric scales. The conversions are based so far as possible upon recent information supplied by the manufacturers of the several instruments.

| Scale. | Equivalent. |
|---|---|
| 1° Ventzke sugar scale | = 0.34657° angular rotation *D*. |
| 1° angular rotation *D* | = 2.88542° Ventzke sugar scale. |
| 1° French sugar scale | = 0.21666° angular rotation *D*. |
| 1° angular rotation *D* | = 4.61553° French sugar scale. |
| 1° French sugar scale | = 0.62516° Ventzke sugar scale. |
| 1° Ventzke sugar scale | = 1.59960° French sugar scale. |
| 1° Wild sugar scale | = 0.13284° angular rotation *D*. |
| 1° angular rotation *D* | = 7.52814° Wild sugar scale. |
| 1° Wild sugar scale | = 0.38329° Ventzke sugar scale. |
| 1° Ventzke sugar scale | = 2.60903° Wild sugar scale. |
| 1° Wild sugar scale | = 0.61313° French sugar scale. |
| 1° French sugar scale | = 1.63098° Wild sugar scale. |

(Normal weight = 26.00 gms. Ventzke scale; 16.29 gms. French scale; 10.00 gms. Wild scale.)

The Ventzke sugar scale is employed upon the Schmidt and Haensch, Peters, and Frič saccharimeters. The French sugar scale is employed upon the Laurent-Jobin and Duboscq-Pellin saccharimeters.

The slight differences in ratio between normal weights and scale equivalents have already been discussed.

# CHAPTER VII

## POLARISCOPE ACCESSORIES

### Illumination of Polariscopes

For the illumination of polariscopes and saccharimeters numerous lamps have been devised and the chemist must be guided in his selection by type of instrument, nature of substance to be polarized, and the kind of light supply available. Before describing the various types of lamps, a word should be said regarding the general subject of illumination.

A much neglected point in the illumination of polariscopes and saccharimeters is the placing of the light at the proper distance from the condensing lens. The light should never be placed so near as to over-heat the metal at the end of the instrument; neglect of this precaution may cause a softening of the balsam and wax mountings of the polarizer and lead to serious derangement of the optical parts.

The proper rule in setting up the polariscope is to place the light in such a position that its image is clearly defined upon the analyzer diaphragm; this is best accomplished by fastening a needle or other sharp-pointed object just before the light and moving the instrument or light until a clear inverted image of the point is obtained upon a piece of white paper placed before the analyzer diaphragm. When the light is thus focused the polariscope is least susceptible to changes in zero point. The proper position of polariscope with reference to light can be seen from Fig. 96, which shows the arrangement of the optical parts in a double-wedge saccharimeter. When correctly placed an inverted magnified image of the light $I$ is obtained at $A$. The reciprocal of the focal distance of the condensing lens will then equal the sum of the reciprocals of the distances of lens from light and of lens from image.

*Example.* — In the case of a Schmidt and Haensch saccharimeter the focal distance of the condensing lens was found to be 5 inches; the distance from lens to analyzer diaphragm was 20 inches; the distance for placing the light would then be $\frac{1}{x} + \frac{1}{20} = \frac{1}{5}$ or $6\frac{2}{3}$ inches from the condensing lens.

The telescope $T$ (Fig. 96) is focused by the observer upon the dividing line of the field at $C$ and the analyzer or compensator turned to the point of even illumination. The dividing line at $C$ will then disappear and the entire field appear of equal intensity. This will be the case even with slight variations in intensity in different parts of the illumination, since at the point $C$, upon which the eye of the observer is focused, the light from any part $p$ of the illumination will be dispersed through different parts of the field (as shown in the figure by the dotted lines); any slight uneveness in the source of illumination will thus be distributed and not noticed by the eye. Great irregularities in illumination, however, must be avoided, and for this reason it

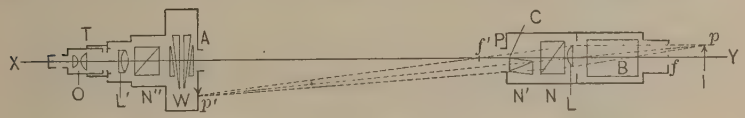



Fig. 96. — Showing method of illuminating polariscopes.

is important that the instrument be kept in perfect alignment with its longitudinal axis at right angle to the source of light. It is best to have instrument and light rigidly fixed. Polariscopes mounted upon trestle supports are preferable to those upon tripods since a slight knock may swing the latter out of alignment and cause a change in the zero point.

Variations in the brightness of illumination are also undesirable and for accurate work the emission of light should be constant. The optical center of gravity of purified sodium light, for example, is 589.22 $\mu\mu$ for a certain average brightness of flame; variations in this brightness, however, may change the wave length by 0.11 $\mu\mu$ with corresponding differences in the rotation of polarized light (25″ for a rotation angle of 20 degrees). With salts of the alkalies and alkaline earths, increasing the brightness of flame (increase of vaporized salt per unit volume of flame) produces an irregular broadening of the spectral lines with a shifting of the mean wave length toward the red end of the spectrum.

**Lamps for Sodium Light.** — Of the various polariscope lamps for sodium light only a few of the more common forms will be described. The lamp shown in Fig. 97 illustrates the essential principles of most sodium lamps. This consists of a Bunsen burner with side entrance for gas at $s$ to prevent stoppage of inlet through dropping of fused salt; the burner is surmounted by a chimney which can be adjusted

to the desired height by the screw $h$. The holder for the fused salt consists of a spoon-shaped bundle of fine platinum wires attached to an upright support and can be moved in and out the flame through a slot in the chimney by means of the screw $p$; the door $k$, which closes the front of the chimney, allows only the brightest section of the flame to shine through and excludes the greater part of the heat. The flame is adjusted so as to be colorless, with as strong an air blast as possible, that the light may be free from incandescent carbon particles.

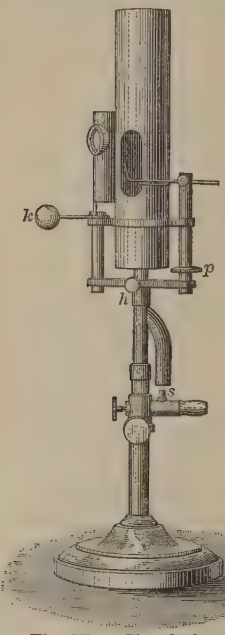


Fig. 97. — Simple form of sodium lamp.

In place of wire holders for the salt many sodium lamps use spoons or V-shaped boats of sheet platinum or nickel, which are in some cases perforated with fine openings. The hot part of the flame impinges upon the spoon and produces a sheet of sodium light upon each side. The fused salt must be renewed as fast as vaporized; a convenient means of effecting this renewal is shown in Přibram's * sodium lamp, Fig. 98, which contains two boats; the empty one is drawn out for refilling and the one in reserve inserted in its place.

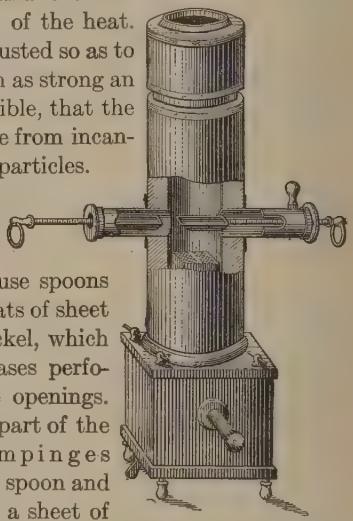


Fig. 98. — Přibram's sodium lamp.

The sodium lamp of Landolt †, Fig. 76, gives a more intense flame than either of the lamps just described. It consists of a powerful Muencke gas burner with cylindrical chimney $L$. Upon the latter are placed two heavy nickel wires supporting rolls of fine nickel wire netting which contains fused salt. The burner is surmounted by a second rectangular chimney of sheet iron with a movable brass door containing apertures of 20, 15, and 10 mm. diameter.

The simplest and cleanest of sodium lamps and the one giving the most continuous flame is that of Zeiss, Fig. 99. This is composed of

* Z. analyt. Chem., **34**, 166. † Z. Instrument., **4**, 390.

an upper part $A$, capping an ordinary Bunsen burner and secured to it by means of a screw. The casting $A$ carries the diaphragm-screen $K$, out of which the rectangular opening $L$ is cut, also the flat burner $C$ producing a square flame, and a small support for the salt carrier $E$, which consists of a piece of pumice stone, measuring about $4 \times 1 \times \frac{1}{2}$ cm., saturated with salt. It is held upon the support by the spring clip $F$ and can be regulated to the flame by means of the screw $J$ operating on the spring $GH$. It is best to adjust the pumice stone so that it merely touches and tinges the flame. If $E$ be too deeply inserted in the flame, the latter is over-cooled and a dark, rather sharply defined zone is produced. The flickering margins of the flame are cut off by the diaphragm $K$. A few minutes are needed for heating the pumice before the flame attains its maximum brilliancy, after which it will remain constant for hours together. The tablets of pumice stone saturated with salt are supplied by the trade at small cost.

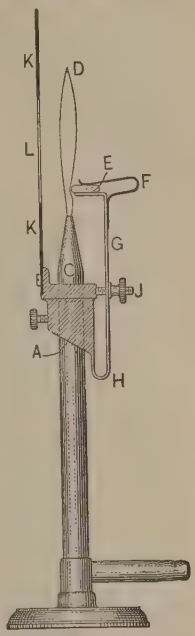


Fig. 99. — The Zeiss sodium lamp.

In place of common salt, sodium bromide is sometimes used for illumination. This gives a much stronger flame, but the vaporization is much more rapid than with salt and there is the additional disadvantage of giving off bromine vapors which may attack the instrument unless the lamp is placed under a hood.

Sodium carbonate, sodium phosphate, sodium nitrite, and mixtures of these with salt in various proportions are also used for sodium lamps. Sticks of fused sodium carbonate heated in an oxygen blast lamp give a flame of great brilliancy, and this is the form of light recommended by Landolt * when intense illumination is desired.

**Purification of Sodium Light.** — For accurate polariscope measurements it is necessary to purify the sodium light from other rays. This can be done either by use of light filters or by spectral separation of the extraneous rays.

Sodium light can be freed from most of the foreign rays at the violet end of the spectrum by means of bichromate solution, which has a strong absorption band in the green and blue. The rays at the other end of the spectrum can be removed by uranous sulphate solution, which has a strong absorption band in the red. A combination of

* "Das optische Drehungsvermögen" (1898), p. 359.

these two solutions, as in the Lippich light filter, constitutes the most effective absorbent means of sodium-light purification known.

*Lippich Light Filter.* — The Lippich light filter consists of a tubular cell closed at the ends by tightly fitting cover glasses and divided by a glass plate into two smaller cells of unequal size. The larger cell, 10 cm. long, is filled with a 6 per cent filtered solution of potassium bichromate, the smaller cell is filled with a solution of uranous sulphate, $U(SO_4)_2$, prepared as follows: 5 gms. of purest uranyl sulphate, $UO_2SO_4 + 3\,H_2O$, are dissolved in 100 c.c. of water, and 2 gms. of powdered chemically pure zinc added; 3 c.c. of concentrated sulphuric acid are then added in 1 c.c. portions, waiting each time until the evolution of hydrogen has nearly ceased; the flask is corked during the reaction, and is allowed to stand about six hours, when the solution is filtered and the cell immediately filled in such a way as to leave only the smallest possible bubble of air behind. After standing for a day the cell is ready for use; the uranous solution retains its stability for one to two months, or until its deep green color is changed by oxidation into the yellow of the uranyl compound, when the cell must be refilled with fresh solution. The weights and volumes prescribed for making up the absorbent solutions must be rigidly adhered to.

The spectrum purification of sodium light by means of glass prisms is the most thorough of all methods of purification. The process, which is a somewhat complicated one, is required, however, for only the finest physical measurements. Landolt gives the following average wave lengths for sodium light from different sources in which the wave length of the $D_1$ line is placed at 589.62 $\mu\mu$ and the $D_2$ line at 589.02 $\mu\mu$.

TABLE XXVI

*Wave Length of Different Kinds of Sodium Light*

| Number. | Source of light. | Purification. | Wave length in $\mu\mu$. |
|---|---|---|---|
| 1 | Bunsen flame with NaBr... | 10 cm. layer of 9 per cent $K_2Cr_2O_7$ in water. | 592.04 |
| 2 | Bunsen flame with NaCl.... | 10 cm. layer of 9 per cent $K_2Cr_2O_7$ in water. | 589.48 |
| 3 | Burner with NaCl or NaBr. | Lippich filter $K_2Cr_2O_7$ and $U(SO_4)_2$. | 589.32 |
| 4 | Sodium light..... | Perfectly spectral pure; light of only the two $D$ lines. | 589.25 |
| 5 | Landolt lamp with NaCl ... | 1.5 cm. layer of 6 per cent $K_2Cr_2O_7$ in water. | 588.94 |
| 6 | Bunsen flame with NaCl.... | 10 cm. layer of 9 per cent $K_2Cr_2O_7$ in water and 1 cm. layer of 13.6 per cent $CuCl_2$ in water. | 588.91 |
| 7 * | Landolt lamp with NaCl ..... | Unpurified | 588.06 |

The Lippich light filter gives a wave length exactly between the two *D* lines of sodium and agreeing very closely with that obtained by spectral purification. In all cases where light filters are used the solutions must be placed between lamp and condensing lens (see Fig. 96).

**Lamps for White Light.** — For illuminating polariscopes and saccharimeters with white light, a large number of lamps have been devised for use with oil, alcohol, gas, acetylene, and electricity.

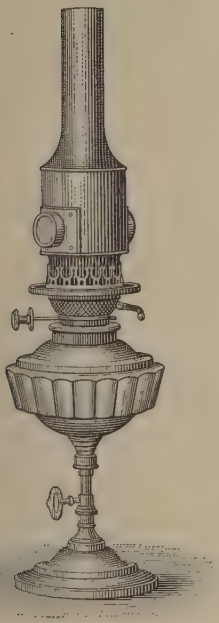


Fig. 100. — Hinks's oil lamp with duplex burner.

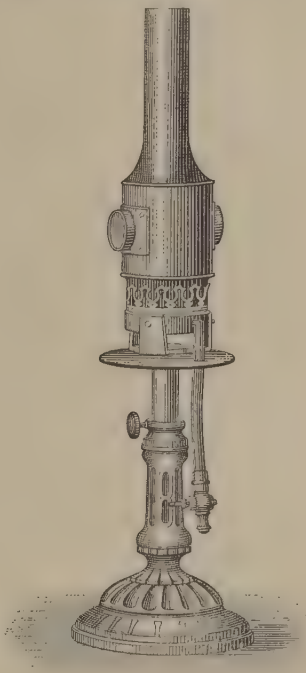


Fig. 101. — Hinks's gas lamp with triplex burner.

A convenient form of oil lamp with duplex burner and adjustable support is that of Hinks, Fig. 100. The Hinks gas lamp with triplex burner is shown in Fig. 101. The metal chimneys of these lamps are usually fitted on the inside with a porcelain reflector; the focusing lens which is sometimes placed in the aperture of the chimney should be removed as it may cause an incorrect passage of the beams of light through the polariscope.

The best forms of gas lamp for illuminating are those provided with an Auer or Welsbach mantle (Fig. 102). The outer cylinder of these lamps, composed of sheet metal or asbestos, contains an opening whose lower half is covered with a plate of ground glass for diffusing the light; the upper uncovered part of the opening serves for illuminating the polariscope scale. A form of lamp for burning alcohol somewhat similar in design to the above is shown in Fig. 103. Gas burners for producing lime or zircon light are also used for illuminating polariscopes. Acetylene lamps of 25 to 50 candle power give a light of great

Fig. 102. — Gas lamp with Welsbach mantle.

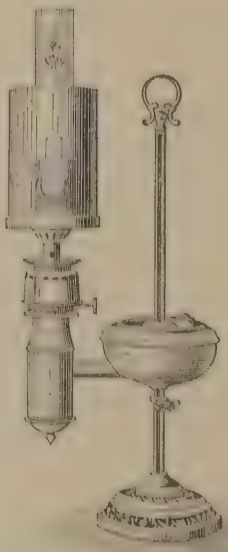


Fig. 103. — Alcohol lamp with Welsbach mantle.

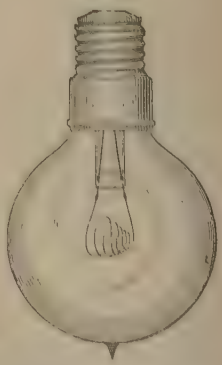


Fig. 104. — Stereopticon electric lamp.

brilliancy and are especially valuable upon sugar plantations where gas or electricity is not available. The acetylene lamps should be fitted with cylinders similar to those in Figs. 100 or 102.

For electrical illumination a stereopticon 32-candle-power incandescent lamp is very suitable (Fig. 104); the closely wound filament concentrates the light within narrow compass, giving great intensity of illumination. These lamps are best mounted in cylinders similar to that in Fig. 102; a plate of ground glass is necessary for diffusing the

light, otherwise the irregularities in source of emission will not be sufficiently equalized for obtaining a uniform field.

A small electric attachment devised by Schmidt and Haensch for illuminating their saccharimeters is shown in Figs. 88 and 105. The

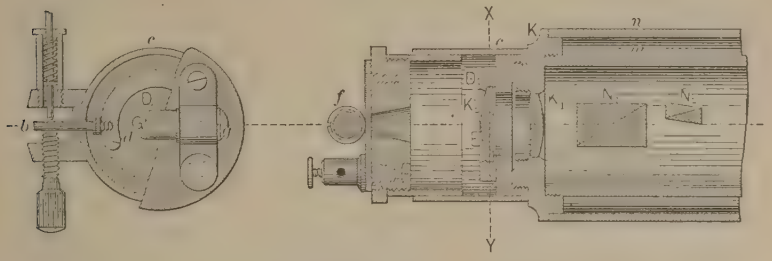



Fig. 105. — Schmidt and Haensch six-volt saccharimeter lamp.

small lamps are adapted for a six-volt current which is supplied by a storage battery or from the main line after reducing the voltage. The apparatus which is controlled by the switch $S$ (Fig. 88) is screwed on the polarizing end of the saccharimeter. The electric lamp is held in position by two spring clips which are in connection with the two terminals. The lenses $K_2$ and $K_1$ (Fig. 105) project the light upon the diaphragm of the analyzer. As the horizontal filament is not always quite concentric to the frame, a vertical adjustment is necessary. To work the adjustment, the ring $D$, which carries the lens $K_2$, is rotated by the screw and projecting arm $b$. If the lamp is also to be used for illuminating the scale of the instrument, the mirror $S'$ (Fig. 88) is set at an angle of 45 degrees, in which position the reflected light is concentrated by the lens $H$ upon the opening $a$ (Fig. 89).

## Polariscope Tubes

For retaining sugar solutions during polarization there are a variety of tubes of different construction, form, and length. In the selection of these the chemist must be guided more or less by the nature of his work. All tubes, however, when accuracy of observation is desired, must conform to three general requirements: (1) the length of the tube must be accurately fixed; (2) the ends of the tube and the surfaces of its cover glasses must be plane parallel; (3) the tube must be centered evenly in its mountings and, when fitted with its caps, should be free from eccentricity. There are other minor requirements of

tube construction which will be given under the description of the different forms.

Fig. 106 shows the most common and simplest forms of glass polarization tubes. These and other forms of tube are usually supplied in lengths of 25 mm., 50 mm., 100 mm., 110 mm., 200 mm., 220 mm., 400 mm., 500 mm., and 600 mm.; for special kinds of work tubes of several meters' length have been constructed.

A tube of 200 mm. length is used for the normal weight of all saccharimeters. If, on account of depth of color, a 100-mm. or 50-mm. tube is employed and the resultant reading is recalculated by multiplying by 2 or 4, there is, of course, a corresponding doubling or quadrupling of the errors of observation; short observation tubes are to be used therefore only in extreme cases. With very dilute sugar solutions

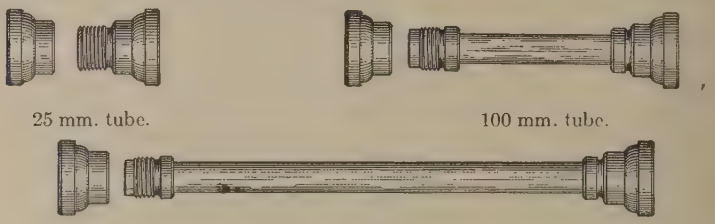



Fig. 106. — Forms of plain glass polariscope tubes.

and with sugars or sugar mixtures of low specific rotation the 400-mm. or 600-mm. tube will increase the accuracy of the observation, provided the color be not too great to disturb the reading. Tubes of odd lengths, such as 55 mm., 110 mm., 220 mm., etc., should be distinctly marked lest they be confused with the 50-mm., 100-mm., and 200-mm. sizes.

**Mounting of Polariscope Tubes.** — The ends of the glass observation tubes are cemented into metal mounts which are threaded for the purpose of receiving the screw cap. Litharge and glycerine make a much better cement than the waxy material employed by most manufacturers. The latter substance, especially on warm days, softens readily and when in this condition there is danger in screwing on the cap of drawing the mount from its setting so that it projects slightly beyond the ends of the tube; the length of the column of liquid to be polarized may thus be increased and a considerable plus error introduced in the observation. The ends of the glass tubes should project only slightly beyond the threaded heads; if too much of the end is exposed there is danger of chipping or breakage. The chemist should not attempt to reset his tubes unless he has a small lathe in which they

can be centered and revolved while the cement is hardening, otherwise the tubes may not be evenly mounted.

A simple means of testing for eccentricity of mounting is to place the tube, with caps screwed on, in the trough of a polariscope and while giving it a rotatory motion to view the opening through the tube with reference to the polariscope field. If the tube has been properly centered and the caps are free from eccentricity the tube opening will remain in the center of the field and show no wabbling movement during rotation. To test for plane parallelism of the ends of the tube and of cover glasses, the experiment just described is repeated with the cover glasses in position and the tube filled with water. If the ends of the tube have not been ground squarely across or the cover glasses are not plane parallel, the opening of the tube will wabble perceptibly during rotation owing to the refraction of light through the water from the inclined surfaces of the cover glasses. A difference of several tenths of a Ventzke degree may be noted between the readings of a tube in different positions through lack of plane parallelism in ends or cover glasses. According to Landolt the angle between the opposite ground-end surfaces of a polariscope tube should always be less than 10 minutes and the angle between the two planes of a cover glass less than 5 minutes. The small angles of inclination between planes of cover glasses and between ends of tubes not exceeding 200 mm. in length is measured by a spectrometer.

Fig. 107.—Landolt's gauge for calibrating polariscope tubes.

**Calibration of Polariscope Tubes.**—A most convenient means of calibrating the length of polariscope tubes is the measuring gauge of Landolt, shown in Fig. 107. This gauge, which has an adjustable handle *c*, consists of a measuring rod *A* of steel graduated for a distance of 400 mm. and provided with a sliding vernier *b* which gives readings to 0.1 mm. The lower end of the rod and the bottom of the vernier are provided with knife edges. When the knife edge of the rod is placed upon a smooth hard surface, such as glass, and the vernier brought down until its knife edges are in close contact with the same surface, the zero point of scale and vernier should agree. If there is lack of agreement, the zero point of the vernier may be either adjusted or the difference noted and applied to all readings. To calibrate an observation tube, one end of the tube is closed with its cover glass and

cap, and after placing in an upright position with the closed end down the measuring rod is inserted until its knife edge touches the cover glass; holding the rod perfectly upright the vernier is slipped down until its knife edges coincide with the upper end of the tube; the reading of the scale and vernier will then give the length of tube. Other readings are made, rotating the rod a little each time from its original position, and the average taken. Calibration of tubes should be made at the standard temperature 20° C.; if measurements are made at temperatures very different from this the changes in length of tube and gauge due to expansion or contraction must be taken into account (coefficient of expansion in length 1° C. for steel = 0.000013 and for glass = 0.000008). Measuring gauges can be verified as to accuracy at the Government Bureau of Standards.

The measuring gauge of Landolt will detect an error of 0.1 mm., which is equivalent to an error of 0.05° V. for a sugar solution polarizing 100° V. in a 200-mm. tube. This is sufficiently close for ordinary saccharimetric measurements; if a finer determination of tube length is desired the measurement must be made upon a comparator; by means of this instrument measurements can be made to 0.01 mm.

**Cover Glasses.** — The cover glasses used upon polariscope tubes must be of strong, colorless, and optically inactive glass; their surfaces must be plane parallel and free from cracks or scratches. In screwing the caps upon observation tubes, care must be taken that no severe pressure is brought to bear upon the cover glasses; otherwise the strain will render the glass optically active and produce serious errors in the observation. If a cover glass is optically active turning the tube in the trough of the polariscope will usually show variations in the intensity of the field with considerable difference in the reading for various positions of the tube. The practice of rotating the observation tube between readings is always a good one; in this way errors due to defective cover glasses, bad washers, pressure of caps, eccentricity, etc., may be detected which would otherwise escape notice. Cover glasses which have been rendered optically active through pressure should not be used for a day, in order that sufficient time may elapse for readjustment to neutrality.

**Washers.** — Another common source of error in polariscopic work are badly fitting rubber washers in the screw caps of the tubes. The washers should be of soft rubber and lie evenly against the back of the cap without the slightest marginal elevation, otherwise the washer in tightening the cap may give the cover glass an inclined position and cause a considerable increase in the reading.

### Special Forms of Polariscope Tubes

*Schmidt and Haensch Tube with Enlarged End.* — Another form of glass polarization tube which presents several advantages is the Schmidt and Haensch tube with one end enlarged (Fig. 108). The enlargement serves as a receptacle for any air bubbles which may be enclosed with the liquid; the retention of a small air bubble in the tube is in fact desirable since, by moving the bubble through the liquid from end to end

Fig. 108. — Schmidt and Haensch polariscope tube with enlarged end. (Air bubbles are collected at the point *a*, outside of the field of vision.)

before reading slight differences in temperature are equalized, and no troublesome striations, due to currents of solution of different temperature, are present to distort the field. Tubes without enlargement must not retain air bubbles with the liquid; if striations are present the tube must remain at rest until the solution has reached equilibrium. The most frequent cause of a striated field is the warming of the solution in the tube by the hand; for this reason tubes should be handled only by the metal caps when placing in the instrument.

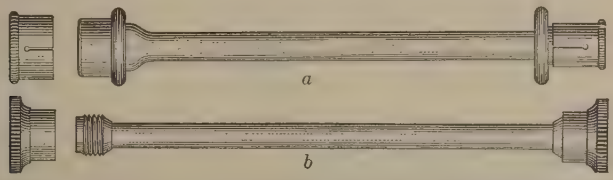



Fig. 109. — (*a*) 200 mm. Landolt polariscope tube with sliding cap and enlarged end; (*b*) 200 mm. metal polariscope tube.

*Landolt's Tube.* — To prevent the liability of excessive pressure upon cover glasses, Landolt has devised a tube with sliding cap, which is shoved into position over the metal mount (Fig. 109a). The French manufacturers also provide a cap that is shoved on and fastened with a bayonet catch. Tubes with screw caps, however, are the ones most preferred and, if care be taken not to draw them up too tightly, will be found to answer all requirements. When observation tubes are used in large numbers it is a great advantage to have all caps interchangeable.

*Metal Polarization Tubes.* — Polarization tubes of brass or nickel or other metal are preferred by many chemists. Such tubes, a form of which is shown in Fig. 109b, have the advantage of greater durability,

but the disadvantage of being susceptible to the attack of acids (as in the method of inversion) or other corrosive liquids. Brass tubes have also more than twice the coefficient of expansion of glass tubes,

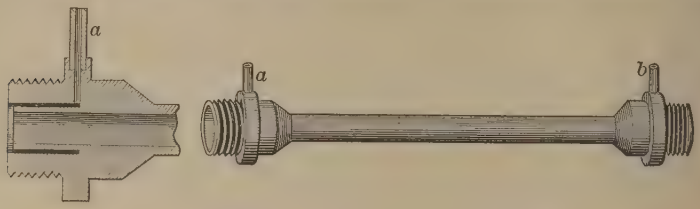



Fig. 110. — Pellet's tube for continuous polarization.

the coefficient ($\beta$) for 1° C. being 0.000008 for glass and 0.000019 for brass. For glass and brass tubes measuring exactly 200 mm. at 20° C., the length at 35° C. ($L_{t^\circ} = L_{20^\circ}[1 + \beta\ (t^\circ - 20)]$) = 200.024 mm. for glass

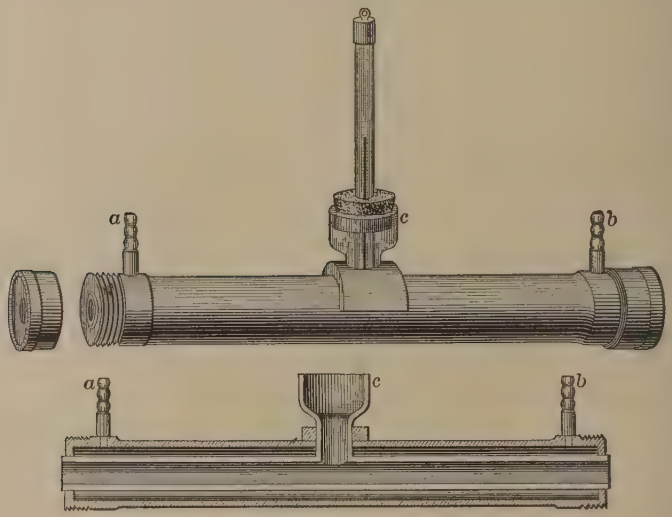



Fig. 111. — Glass polarization tube with metal jacket.

and 200.057 mm. for brass, errors in length of no great significance. A more serious objection against metal tubes is the danger of their being bent out of alignment through hard or long usage. A knock or fall may cause a metal tube no apparent injury yet may bend it sufficiently to produce a considerable error in the polariscope reading. A number of brass polariscope tubes, recently submitted to the author for examina-

tion, were so badly out of alignment that rotating the tubes in the trough of the polariscope caused a difference of over 0.2° V. in the reading.

*Pellet's Tube for Continuous Polarization.* — In the polarization of a large number of solutions in succession, as in the analysis of sugar beets, juices, etc., the Pellet tube for continuous polarizations is often of great use. Sections of this tube, which is made of metal, are shown in Fig. 110. The ends of the tube are closed and after placing in the instrument the solution to be polarized is poured through a small funnel into one of the nipples, *a* or *b*, the excess escaping through an exit tube connected by rubber tubing to the nipple at the opposite end. As soon as the solution is polarized, the succeeding solution is poured into the tube; the disappearance of striations and the clearing of the field indicate when the previous solution has been completely displaced. The Pellet tube will accomplish a valuable saving of time in certain kinds of work, but it is usually advisable to limit its use to sugar solutions of approximately the same density; to displace a concentrated sugar solution with one that is exceedingly dilute, or vice versa, is attended with more or less risk of error.

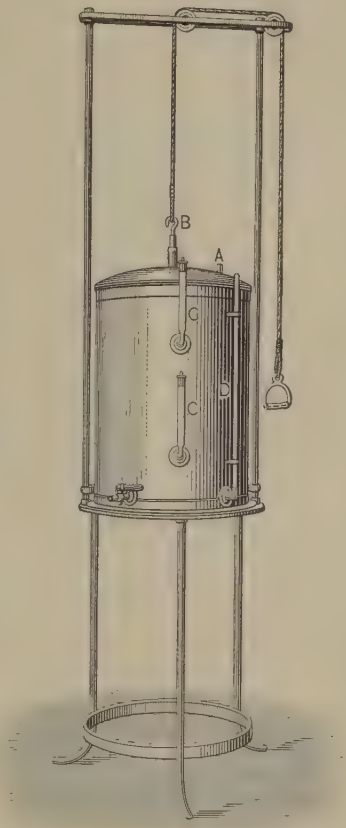



Fig. 112. — Reservoir for supplying water of constant temperature.

*Polarization Tube with Metal Jacket.* — For polarizing sugar solutions, where the temperature must be measured or controlled, a jacketed observation tube such as shown in Fig. 111 is recommended. This consists of an inner tube of glass or metal with a central opening, *c*, which can be used for filling and for inserting a thermometer; an outer mantle of brass or nickel surrounds the inner tube and is provided with nipples for inlet and exit of hot or cold water as may be desired.

For supplying water of constant temperature for observation tubes, the Zeiss apparatus described on page 59 may be used. A form of water supply reservoir with stirrer, recommended by Landolt,* is shown in Fig. 112. The reservoir, which is insulated, is filled through the opening *A* with water to the desired level, indicated by the tube *D*. The water is heated by means of a burner to the desired temperature, shown by the thermometers at *C*, the heat being equalized by raising and lowering the stirrer *B*.

A form of constant temperature bath designed by Hudson † is shown in Fig. 113. The mechanical stirrer not only secures an even temperature through the bath, but also acts as a rotary pump which

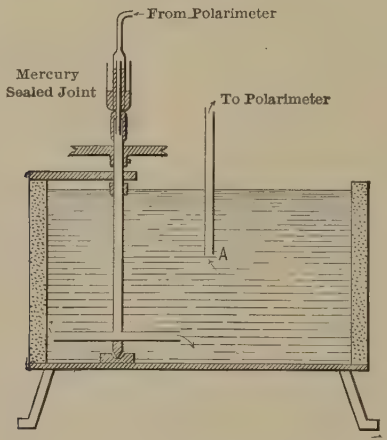


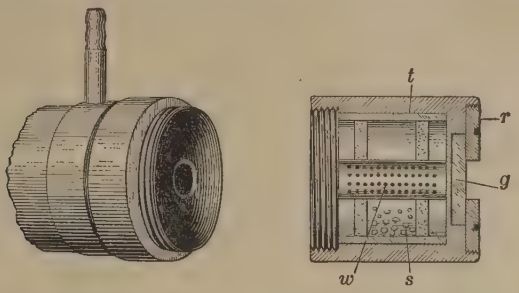

Fig. 113. — Hudson's constant temperature water-bath.

creates a constant circulation of water as shown by the direction of the arrows.

*Wiley's Desiccating Caps.* — When solutions are polarized at temperatures below the dew point of the atmosphere, the cover glasses of the observation tube must be protected against condensation of moisture by means of desiccating caps such as designed by Wiley ‡ (Fig. 114). These are generally made of some non-conducting material such as hard rubber; they are closed at the end with a tightly fitting cover glass and contain a tube for holding calcium chloride or other desiccating substance.

* "Das optische Drehungsvermögen" (1898), pp. 397.
† Hudson, J. Am. Chem. Soc. **30**, 1572. ‡ J. Am. Chem. Soc. **18**, 81.

When solutions are polarized at very high temperatures as at 87° C. (the point of inactivity for invert sugar) the use of glass, unless carefully annealed, for the inner tube of the water jacket is precluded. Polariscopic work at high temperature is generally performed in




(I) (II)

Fig. 114. — (I) Threaded cap of polariscope tube. (II) Dessicating cap which screws on over threads of (I); *t*, removable glass tube containing dessicating substance *s*; *w*, inner perforated metal tube; *g*, cover glass held in position by threaded disk *r*; the disk is unscrewed by inserting a spanner in the two holes marked in black.

jacketed tubes constructed entirely of brass or nickel, the inner surface of which has been gold plated. The length of a 200-mm. tube (20° C.) at 87° C would be 200.107 mm. for glass and 200.255 mm. for brass, equivalent to a plus error of 0.054° V. and 0.128° V. respectively for solutions polarizing 100° V. in a 200-mm. tube.

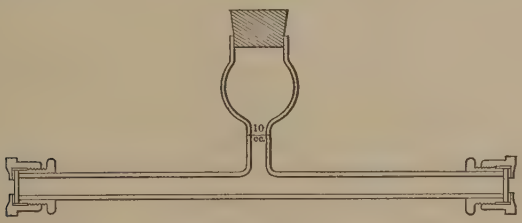


Fig. 115 — Yoder's volumetric polariscope tube.

*Yoder's Volumetric Polariscope Tube.* — A volumetric polariscope tube is convenient for certain kinds of saccharimetric work. A tube of this description, designed by Yoder, is shown in Fig. 115.

The capacity of the tube to the graduation mark upon the neck is 10 c.c. By varying the length and diameter the tubes can be adjusted to any convenient volume.

### Balances for Polariscopic Work

For the operations of weighing in saccharimetric work three types of balances are required, an analytical balance, a so-called sugar balance, and a balance for coarse weighing.

The analytical balance should have a capacity of 200 gms. and with this load be sensitive to 0.1 mg. Such a balance is required for all analytical processes, for determination of specific rotations, for calibration of flasks, weighing of pycnometers, and all other operations where accuracy is essential. A balance of the type shown in Fig. 17 will answer for this purpose. With this balance a set of accurate analytical weights (including one 100 gms. weight) will be needed.

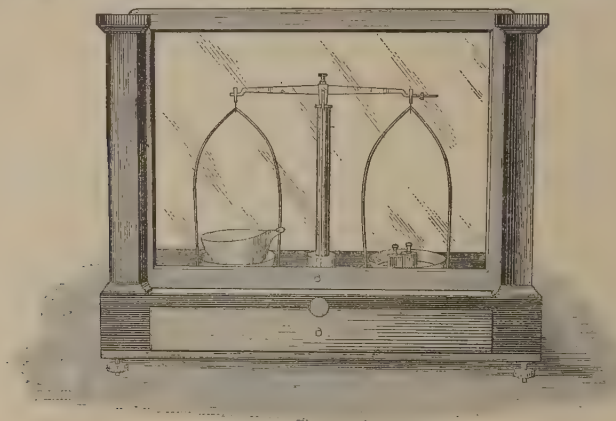


Fig. 116. — Sugar balance.

In addition to the above a less delicate balance, sensitive to 2.5 mgs. with a load of 250 gms., will be required for the rapid weighing of definite amounts of sugar, molasses, and other products for ordinary saccharimetric work. For saccharimeters employing a normal weight of 26 gms., 0.01 degree Ventzke corresponds to 0.0026 gm. sucrose in 100 true cubic centimeters. Since the majority of saccharimeters can be read only to 0.05° V it is evident that weighing within 5 mgs. is sufficiently accurate for ordinary purposes of saccharimetry. The weighing out of normal weights of sugar, etc., for saccharimeters should not be done upon an analytical balance; the errors due to evaporation from moist substances during the slower adjustment of the analytical balance will usually exceed any advantage in greater accuracy of weight. A so-called " sugar balance " of the type shown in Fig. 116

answers very well for this kind of work. This balance may also be used for the weighing out of chemicals for making up solutions of reagents. A set of second quality weights should be provided for approximate weighing, and also the normal weights belonging to the saccharimeter.

The Mohr cubic centimeter normal and half-normal weights (26.048 gms. and 13.024 gms.) are usually furnished in a cylindrical form and the true cubic centimeter weights (26.000 gms. and 13.000 gms.) in a cubical form (Fig. 123), the shape of the weight thus guarding against

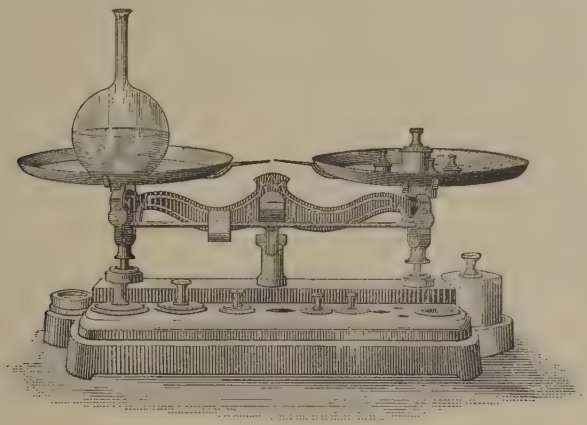


Fig. 117. — Metric solution scale.

confusion. Normal weights, which are in constant use, should be tested frequently upon the analytical balance against losses in weight through wear. If a deficiency exceeding 1 mg. is noted, the stem of the weight should be unscrewed and a small piece of tin or aluminum foil be placed in the cavity sufficient to bring the weight up to its proper value.

In addition to the two balances just described a heavy balance or scale for weighing out material in bulk, preparing large quantities of reagents, etc., will be required. A metric solution scale with sliding counterpoise such as is shown in Fig. 117 is very good for this purpose. A set of third quality weights up to 5 kgs. should also be provided for coarse weighings.

### Flasks for Polariscopic Work

For the preparation of sugar solutions in polarimetric and saccharimetric work various flasks have been devised of different shape and construction.

**Flasks for Solution by Weight.** — When sugar solutions are made up according to percentage a glass-stoppered flask of the form shown as No. VI in Fig. 118 is recommended. The flask, which is supplied in many sizes, need not be graduated. Before using, it is thoroughly cleansed and dried, and then weighed. The approximate quantity of substance to be examined is then transferred to the flask and after stoppering the latter is reweighed. The approximate amount of distilled water or other solvent is then added and the flask and contents reweighed as before. The percentage of substance in solution is then readily calculated from the weight of substance taken and the combined weights of substance and solvent. The flask should not be filled too full; sufficient space should be left for gentle rotation of the liquid while effecting solution. The flask should always be kept stoppered to prevent evaporation.

*Reduction of Solution Weights to Vacuo.* — For very accurate physical measurements the weights taken in air must be reduced to vacuo, since a substance weighed in any medium loses in weight an amount equal to that of the medium displaced. If $W$ is the true weight of a substance of density $D$, in vacuo, then the volume of substance is $\frac{W}{D}$, and if $s$ is the density of the air at the time of weighing, the loss in weight of the substance in air will be $\frac{sW}{D}$. Similarly if $P$ is the value of the weights in vacuo and $d$ is the density of their material then the loss of the weights in air will be $\frac{sP}{d}$. The equilibrium upon the pans of the balance between substance and weights in air will then be represented by the equation

$$W - \frac{sW}{D} = P - \frac{sP}{d},$$

whence

$$W = P \frac{1 - \frac{s}{d}}{1 - \frac{s}{D}}.$$

The mean value 0.0012 gm. may be taken as the weight of 1 c.c. of air without sensible error. When brass weights are used ($d = 8.4$), the weights in vacuo of glass, water and sugar are found as follows: for glass ($D = 2.5$) $W = 1.000337\ P$, for water 20° C. ($D = 0.998234$) $W = 1.001061\ P$, for cane sugar ($D = 1.59$), $W = 1.000612\ P$. The following example will illustrate the method of application.

| | |
|---|---|
| Weight of flask + sugar in air | 35.2326 gms. |
| Weight of flask alone in air | 25.1240 gms. |
| Weight of sugar in air | 10.1086 gms. |
| Weight of sugar in vacuo = 10.1086 × 1.000612 = | 10.1148 gms. |
| Weight of flask + sugar + water in air | 95.3055 gms. |
| Weight of flask + sugar in air | 35.2326 gms. |
| Weight of water in air 20° C. | 60.0729 gms. |
| Weight of water in vacuo = 60.0729 × 1.001061 = | 60.1366 gms. |
| Weight of sugar + water in vacuo = | 70.2514 gms. |

Per cent sugar in solution from weights in air = 14.403 per cent.
Per cent sugar in solution from weights in vacuo = 14.398 per cent.

It will be noted that the difference is exceedingly slight, so that weighing in air is sufficiently exact for all operations except those demanding extreme accuracy.

**Volumetric Sugar Flasks.** — When solutions of dissolved sugars are made up to a definite volume before polarization, a carefully calibrated volumetric flask must be used; such flasks are supplied in a variety of forms and sizes. If solutions are polarized immediately after making up to volume, as is usually the case, it is not essential that the flask be fitted with a glass stopper.

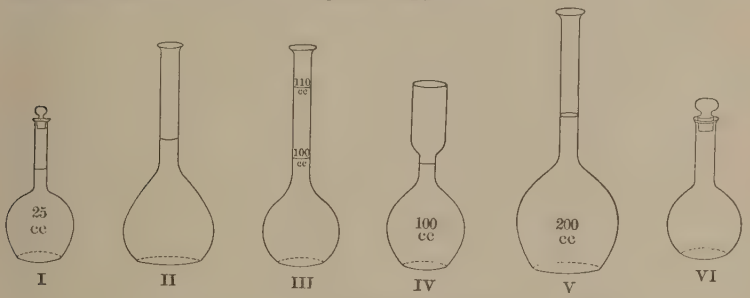



Fig. 118. — Types of flasks for polariscopic analysis.

Volumetric flasks for sugar work are made in 10-c.c., 20-c.c., 25-c.c., 50-c.c., 100-c.c., 200-c.c., and 250-c.c. sizes; 500-c.c. and 1000-c.c. flasks are also occasionally used. For certain kinds of work, where volume of insoluble matter is allowed for, flasks of irregular capacity are used, as 100.6-c.c., 201.2-c.c., etc., for polarization of sugar-beet pulp.

A few of the more ordinary forms of sugar flask are shown in Fig. 118. These may be obtained of any desired capacity. Small sized stoppered flasks similar to No. I are convenient for preparing solutions when small amounts of substance are available. Kohlrausch's sugar

flask (No. IV) with enlarged top is convenient for transferring substances and is in many ways a most desirable flask; it can be obtained in the small sizes and, if desired, with ground-glass stopper. Sugar flasks with double graduation (No. III) for one-tenth dilution are useful for the methods of inversion; they are supplied in 25–27.5-c.c., 50–55-c.c., 100–110-c.c., and 200–220-c.c. sizes.

**Specifications for Sugar Flasks.** — In the selection of sugar flasks the following requirements of the United States Bureau of Standards for volumetric flasks will be found useful.

"The cross section of all flasks must be circular throughout and the neck must merge into the body of the flask so gradually that there will be no hindrance to uniform drainage. Flasks that are manifestly fragile or otherwise defective in construction will be rejected. The part on which the graduation mark is placed must be transparent, of uniform thickness, and free from striæ. The graduation mark must be placed not less than 6 cm. from the upper end and not less than 2 cm. from the lower end of the neck of a flask larger than 100 c.c., and not less than 3 cm. from the upper end or 1 cm. from the lower end of the neck of a flask not larger than 100 c.c. The graduation mark must extend entirely around the neck. The bottom of the flask must be slightly reëntrant, and the flask must be of such form that drainage can take place from the whole interior surface at the same time. The neck of a flask must be perpendicular to a plane tangent to the bottom. The flask must stand solidly when placed on a horizontal plane."

A very desirable 100-c.c. flask for saccharimetric work is that shown in No. II, Fig. 118, and in Fig. 123 designed for use in the Custom-House Laboratories of the United States Treasury Department. The pear-shaped body with its low center of gravity gives the flask greater stability than a spherical form. According to the regulations of the Treasury Department "the flasks shall have a height of 130 mm.; the neck shall be 70 mm. in length and have an internal diameter of not less than 11.5 mm. and not more than 12.5 mm. The upper end of the neck shall be flared, and the graduation marks shall be not less than 30 mm. from the upper end and 15 mm. from the lower end of the neck." With this size of flask the base of the thumb can cover the mouth and the fingers of the same hand easily enclose the bottom — a feature of great convenience when mixing the contents after making up to volume.

**Calibration of Sugar Flasks.** — Sugar flasks are graduated to contain 100 true cubic centimeters at 20° C. or 100 Mohr cubic centimeters at 17.5° C. and should be calibrated before using in the follow-

ing manner. The flask to be tested is first thoroughly cleaned and dried, then weighed empty at the temperature of standardization, and then again when filled to the mark with distilled water at the standard temperature. The distilled water should be boiled just before using, in order to expel dissolved air, and then cooled. Special care is necessary in adjusting the meniscus to the graduation mark; the lowest point of the curve when viewed against a white surface should just touch the level of the graduation mark, the latter appearing to the eye in proper position as a straight line and not as an ellipse. Fig. 119 indicates the proper method of adjustment. The inside of the neck above the meniscus should be wiped perfectly dry with filter paper before reweighing; air bubbles should not be allowed to adhere to the walls of the flask during calibration.

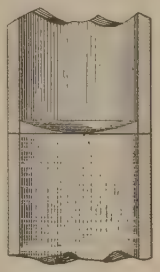


Fig. 119.—Showing proper adjustment of meniscus.

Volumetric 100-c.c. sugar flasks graduated according to the Mohr system should contain 100 gms. of distilled water at 17.5° C., when weighed in air against brass weights; 100-c.c. flasks graduated according to true cubic centimeters should contain 100 gms. of distilled water at 4° C. when weighed in vacuo or 99.7174 gms. at 20° C. when weighed in air with brass weights. (Weight in vacuo of 100 c.c. water at 20° C. is 99.8234 gms. and weight in air (p. 164) is $99.8234 \div 1.001061 = 99.7174$ gms.) The grams of water contained by the flask at 20° C. plus the correction 0.282 will give the volume in true cubic centimeters.

The limits of error allowed by the United States Bureau of Standards for volumetric flasks are the following:

| Capacity. | Limit of error. |
|---|---|
| c.c. | c.c. |
| 2000 | 0.5 |
| 1000 | .3 |
| 500 | .15 |
| 250 | .1 |
| 200 | .1 |
| 100 | .08 |
| 50 | .05 |
| 25 | .03 |
| 10 | .01 |

The limit of error allowed above for 100-c.c. flasks is, however, too high; the error of graduation should not exceed 0.05 c.c. and careful manufacturers can conform to this requirement without trouble. A

lot of 200 sugar flasks purchased by the New York Sugar Trade Laboratory showed the following errors upon calibration.

| Error in volume. | Number of flasks. | Percentage. |
|---|---|---|
| Between 0.00 c.c. and 0.01 c.c. .. | 65 | 32.50 |
| Between 0.01 c.c. and 0.02 c.c. .. | 56 | 28.00 |
| Between 0.02 c.c. and 0.03 c.c. .. | 43 | 21.50 |
| Between 0.03 c.c. and 0.04 c.c. .. | 27 | 13.50 |
| Between 0.04 c.c. and 0.05 c.c. .. | 7 | 3.50 |
| Between 0.05 c.c. and 0.06 c.c. .. | 2 | 1.00 |
| | 200 | |

It is seen that 99 per cent of the flasks were correct within 0.05 c.c. and that over 95 per cent were correct within 0.04 c.c.

### Funnels and Cylinders

In filtering sugar solutions for polarization short-stemmed funnels and cylinders of any of the forms shown in Fig. 120 will be found con-

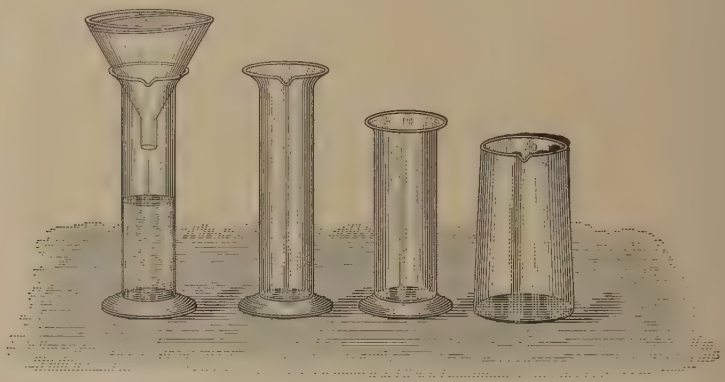



Fig. 120. — Types of cylinders for polariscopic analysis.

venient. The funnels and filters should be of sufficient size to retain 100 c.c. of solution; they should be covered with large watch glasses during filtration to prevent evaporation. Tall narrow filtering cylinders (Nos. I and II, Fig. 120) are preferred by some chemists for the reason that the least surface of filtered liquid is exposed to evaporation. The small-lipped filtering jars (No. IV, Fig. 120) are more convenient, however, for filling tubes, and if covered by funnels and watch glasses will

not allow sufficient evaporation, during the necessary time of filtration, to cause any appreciable error in the polariscope reading.

### Mounting of Polariscopes and Care of Apparatus

If the circumstances permit, polariscopes should always be mounted in a separate room or compartment, where there is no danger of corrosion from the action of fumes or vapors. The polarizing compartment should be well ventilated and easily darkened; lamps and burners for illumination should be placed upon the opposite side of a wall or partition.

Fig. 121. — Cabinet for constant temperature polarization (New York Sugar Trade Laboratory).

In the New York Sugar Trade Laboratory the polariscope cabinet (Fig. 121) constitutes a section of the constant-temperature room. The roof of the cabinet is composed of shutters, for regulating the downward passage of cool air, and the sides of the cabinet are enclosed by dark curtains, which, when drawn, leave a space of one foot at the bottom. This arrangement allows free circulation of air, and the presence of several observers in the cabinet does not affect the temperature.

Where room is not available for a separate compartment, the polariscopes may be mounted in a large box in a dark corner of the laboratory as shown in Fig. 122.

The table supporting polariscopes should be of solid construction. By placing the table upon rubber cushions and setting the polariscopes upon rubber mats, vibration of the instruments and consequent disturbance of the zero point will be largely obviated.

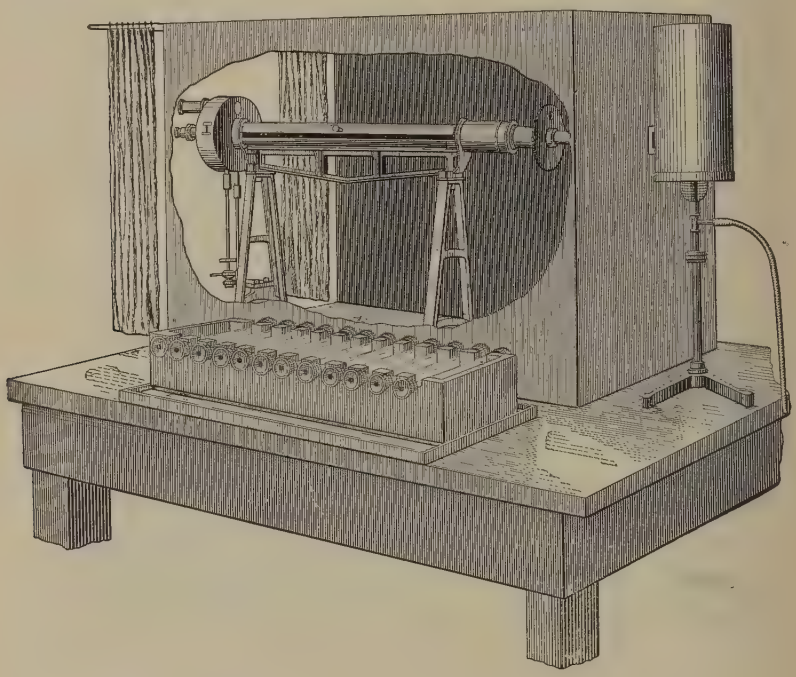


Fig. 122. — Portable polariscope cabinet with section of side removed.

It is essential in saccharimetric work that all apparatus be kept scrupulously clean. The more delicate optical parts of polariscopes, such as polarizer, analyzer, and quartz compensation, are enclosed, in the most modern apparatus, in dust-proof housings, and very rarely require to be disturbed. The diaphragm glasses ($A$ and $P$, Fig. 96) at each end of the polariscope trough are the parts which require most attention. Drops of solution, accidentally adhering to the polariscope tubes, are occasionally splashed against the diaphragm glasses. The

diaphragms, which either screw or slide into position, should be examined frequently and the glasses wiped free of dirt and dust particles. A paper napkin will be found very suitable for cleaning diaphragm glasses, eye pieces, and other exposed optical parts.

The troughs of polariscopes in the hasty round of routine frequently become soiled from contact with wet tubes or spilled liquid. They should be wiped frequently with a damp cloth and the metal surface should be kept smooth and clean.

The bichromate cell should be examined frequently, and the solution replenished as soon as bubbles begin to form, otherwise their appearance may obscure the field.

When the polariscope is not in use, the trough should be closed and the instrument kept covered.

Strict cleanliness must also be observed in the use of polariscope tubes, flasks, and other accessories. In handling and carrying observation tubes a portable rack of the form shown in Fig. 122 will be found convenient.

Where sugar solutions are clarified with lead subacetate, the walls of flasks, cylinders, funnels, and tubes become coated in time with a thin white film of lead carbonate. A good solvent for this coating is a warm solution of sodium hydroxide and Rochelle salts, such as is used in preparing Fehling's solution. Hydrochloric or nitric acid may also be used for removing the deposit. After thorough rinsing in clean water, tubes, flasks, funnels, and cylinders should be allowed to drain and dry upon racks.

# CHAPTER VIII

## SPECIFIC ROTATION OF SUGARS

In the previous chapters the principles which underlie the construction and operation of polariscopes were described; it is now desired to study the application of these principles to some of the problems of sugar analysis.

The polarizing power of a sugar is expressed as specific rotation, or specific rotatory power, by which is meant the angular rotation which a calculated 100-per cent solution, 1-dcm. long, gives to the plane of polarized light. The specific rotation, indicated by the expression $[\alpha]$ can easily be calculated from the angular rotation $a$ of the solution of substance by means of the equation $[\alpha] = \frac{100\,a}{c \times l}$, in which $c$ is the concentration of substance (grams per 100-c.c. solution) and $l$ the length of the observation tube in decimeters. Instead of the foregoing we may use the equation $[\alpha] = \frac{100\,a}{p \times d \times l}$, in which $p$ is the percentage of substance in solution (parts by weight in 100 parts by weight of solution) and $d$ is the specific gravity of solution. ($p \times d = c$ in previous equation.)

The angular rotation, as shown below, depends upon the wave length of the light employed. Sodium light is the illumination most used for polariscopic measurements and as the bright yellow line of sodium is designated the $D$ line of the solar spectrum, the expression $[\alpha]$ for sodium light is written $[\alpha]_D$. Specific rotation for the mean yellow ray $j$ (now no longer used) is written $[\alpha]_j$. The temperature at which the specific rotation is taken is also usually affixed. Thus: the symbol for specific rotation using sodium light at 20° C. is written $[\alpha]_D^{20}$.

The method of calculating specific rotation may best be understood by an example; 20 gms. of cane sugar dissolved to 100 c.c. gives an angular rotation for sodium light of +53.2 degrees in a 400-mm. tube at 20° C. Substituting these values in the equation $[\alpha] = \frac{100\,a}{c \times l}$, we obtain $[\alpha]_D^{20} = \frac{100 \times 53.2}{20 \times 4} = +66.5$ the specific rotation of sucrose for the given concentration.

To calculate specific rotation from the reading of a saccharimeter, the scale divisions of the latter must first be converted to angular degrees by means of the appropriate factor. Thus: 15 gms. of sucrose dissolved to 100 metric cubic centimeters gave a reading of + 57.7 in a 200-mm. tube using a Ventzke scale quartz-wedge saccharimeter. Since 1° V = 0.34657 angular degrees (page 145) then

$$[\alpha]_D \text{ sucrose} = \frac{100\ (0.34657 \times 57.7)}{15 \times 2} = +66.6.$$

### Effect of Kind of Light upon Specific Rotation of Sugars.

Mention has been made of the influence of wave length of light upon specific rotation. In Table XX a comparison was given of the rotations of quartz and sucrose for light of different wave lengths and it was shown that as the wave length decreases the polarizing power of sucrose increases. In the following table the specific rotations of nine different sugars are given for light of different wave lengths in the red, yellow, green, blue, indigo, and violet parts of the spectrum, according to recent measurements by Grossmann and Bloch.* The specific rotations for yellow sodium light, $[\alpha]_D$, the standard values of comparison, are printed in heavier type.

| Sugar. | Concentration, gms. 100 c.c. | Red (r) 656 μμ. | Yellow (y) 589 μμ. | Green (g) 535 μμ. | Blue (b) 508 μμ. | Indigo (i) 479 μμ. | Violet (v) 447 μμ. | Dispersion coefficient $\frac{v}{r}$. |
|---|---|---|---|---|---|---|---|---|
| Xylose..... | 0.866 | + 13.28 | **+ 18.19** | + 21.08 | + 24.50 | + 27.70 | + 31.94 | 2.41 |
| Rhamnose.. | 6.948 | + 7.08 | **+ 8.37** | + 10.27 | + 11.11 | + 12.84 | + 14.38 | 2.03 |
| Galactose .. | 5.603 | + 60.80 | **+ 80.72** | + 99.63 | +116.76 | +131.84 | +152.90 | 2.51 |
| Glucose..... | 4.500 | + 41.89 | **+ 52.76** | + 65.35 | + 73.61 | + 83.88 | + 96.62 | 2.30 |
| Fructose.... | 4.500 | − 76.39 | **− 90.46** | −107.21 | −136.85 | −151.11 | −166.55 | 2.18 |
| Sucrose..... | 4.275 | + 53.18 | **+ 66.50** | + 82.25 | + 91.53 | +104.24 | +121.63 | 2.29 |
| Lactose..... | 2.000 | + 39.82 | **+ 52.42** | + 62.09 | + 72.25 | + 83.25 | + 98.17 | 2.47 |
| Maltose..... | 6.021 | +111.00 | **+137.04** | +166.11 | +176.26 | +227.12 | +233.36 | 2.10 |
| Raffinose... | 3.713 | + 79.63 | **+105.20** | +131.71 | +150.75 | +163.77 | +188.55 | 2.37 |
| | | | | | | | Average | 2.296 |

It is seen that of the nine sugars galactose shows the greatest and rhamnose the smallest dispersion coefficient, the average value 2.296 being the same as that of sucrose and of glucose.

Various formulæ have been proposed for expressing the relationship between specific rotation and wave length of light. Stefan † gives for sucrose the formula $[\alpha] = \frac{2538}{\lambda^2} - 5.58$, in which the wave length $\lambda$ is

* Z. Ver. Deut. Zuckerind., **62**, 19. † Ber. Wiener Akad., **52**, 486.

expressed in ten-thousandths of a millimeter $\left(\frac{\mu\mu}{100}\right)$. The results as thus calculated have only an approximate value, as other factors, such as temperature, concentration, etc., are not considered.

The specific rotations of the different sugars also vary according to the concentration of solution, the temperature of observation and the nature of the solvent. The following table gives the approximate values for the specific rotation of a number of sugars. The effect of concentration and temperature in increasing or lowering the specific rotation is indicated by the direction of the arrow in the respective columns.

TABLE XXVII

*Showing Effect of Increase in Concentration and Temperature upon Specific Rotation of Sugars*

| Sugar. | $[\alpha]_D^{20°}$. | Increase in concentration −0+ | | Increase in temperature −0+ | |
|---|---|---|---|---|---|
| Arabinose | +104.5 | | ? | | ← |
| Xylose | +19.0 | | → | | → |
| Rhamnose | +8.5 | | ? | | ← |
| Galactose | +80.5 | | → | | ← |
| Glucose | +52.5 | | → | | ? |
| Fructose | −92.5 | ← | | → | |
| Invert sugar | −20.0 | ← | | → | |
| Sucrose | +66.5 | | ← | | ← |
| Lactose | +52.5 | | ? | | ← |
| Maltose | +138.5 | | ← | | ← |
| Raffinose | +104.5 | | ? | | ? |

## EFFECT OF CONCENTRATION UPON SPECIFIC ROTATION OF SUGARS

The effect of varying concentration upon the specific rotation of sugars has been studied by many observers and the results of their observations have been expressed in the form of equations. The method of deriving these equations, which is due to Biot,* is of considerable importance to the sugar chemist and deserves to be briefly considered.

**Concentration Equations.** — If the specific rotations of a substance for different concentrations be laid off upon a diagram, in which the specific rotations represent the ordinates and the percentages of substance in solution the abscissæ, the line which connects the several points, will be either a straight line, a section of a parabola, hyperbola, or other curve, or a combination of any two or more of these. Calling the percentage of sugar in solution $p$, the specific rotation can be represented as follows: according to the well-known algebraic equations.

* Ann. chim. phys. [3], **10,** 385; **11,** 96; **28,** 215; **36,** 257; **59,** 219.

I. For the straight line $[\alpha] = a + bp$.

II. For the parabola $[\alpha] = a + bp + cp^2$.

III. For the hyperbola $[\alpha] = a + \frac{bp}{c + p}$.

Having plotted and determined the nature of the curve it remains to calculate the values of the constants $a$, $b$, and $c$ in the above equations. The method of doing this (the method of least squares) is simple, although the work of calculation is somewhat laborious. The following example is given as an illustration:

From the average results of observations by Tollens, Thomson, Schmitz, Nasini, and Villavecchia, the following specific rotations of sucrose were found for different concentrations; 10 per cent + 66.56, 20 per cent + 66.52, 30 per cent + 66.41, 40 per cent + 66.27, 50 per cent + 66.06. An equation is desired for the specific rotation of sucrose for any concentration within these limits.

By plotting the above observations a curved line is obtained presumably a parabola. (In calculating the concentration curves for the specific rotation of sugars the hyperbola is but little used.) Substituting the results in the previous equation II for the parabola we obtain the following:

| | | |
|---|---|---|
| 1. | | $a + 10\,b + 100\,c = 66.56.$ |
| 2. | | $a + 20\,b + 400\,c = 66.52.$ |
| 3. | | $a + 30\,b + 900\,c = 66.41.$ |
| 4. | | $a + 40\,b + 1600\,c = 66.27.$ |
| 5. | | $a + 50\,b + 2500\,c = 66.06.$ |
| Average: I. | | $a + 30\,b + 1100\,c = 66.364.$ |

From the above equations we obtain by subtraction the following:

| | | |
|---|---|---|
| 6. | (5 − 1) | $40\,b + 2400\,c = -0.50.$ |
| 7. | (5 − 2) | $30\,b + 2100\,c = -0.46.$ |
| 8. | (5 − 3) | $20\,b + 1600\,c = -0.35.$ |
| 9. | (5 − 4) | $10\,b + 900\,c = -0.21.$ |
| 10. | (4 − 1) | $30\,b + 1500\,c = -0.29.$ |
| 11. | (4 − 2) | $20\,b + 1200\,c = -0.25.$ |
| 12. | (4 − 3) | $10\,b + 700\,c = -0.14.$ |
| 13. | (3 − 1) | $20\,b + 800\,c = -0.15.$ |
| 14. | (3 − 2) | $10\,b + 500\,c = -0.11.$ |
| 15. | (2 − 1) | $10\,b + 300\,c = -0.04.$ |
| Average: II. | | $20\,b + 1200\,c = -0.25.$ |

By combining equations 6 to 15 into two series and subtracting we obtain the following:

| | | |
|---|---|---|
| III. | (7 + 8 + 10 + 12 + 14) | $100\,b + 6400\,c = -1.35$ |
| IV. | (6 + 9 + 11 + 13 + 15) | $100\,b + 5600\,c = -1.15$ |
| | | $800\,c = -0.20$ |
| | | $c = -0.00025.$ |

Substituting the value for $c$ in equation II we obtain $b = 0.0025$, and substituting these values for $b$ and $c$ in equation I we obtain $a = 66.564$. Substituting these values in the original equation for the parabola we obtain:

$$[\alpha]_D^{20} = 66.564 + 0.0025\ p - 0.00025\ p^2.$$

The calculated specific rotation of sucrose for various concentrations according to the above equation is as follows: 10 per cent 66.56, 20 per cent 66.51, 30 per cent 66.41, 40 per cent 66.26, 50 per cent 66.06, results which agree perfectly with the average observations taken.

The above equation for the specific rotation of sucrose does not hold, however, for concentrations below 10 per cent or above 50 per cent. Tollens* from observations upon 19 solutions ranging from 3.8202 per cent to 69.2144 per cent sucrose calculated the following equations:

For $p =$ 4 to 18 per cent sucrose,

$$[\alpha]_D^{20} = 66.810 - 0.015553\ p - 0.000052462\ p^2.$$

For $p =$ 18 to 69 per cent sucrose,

$$[\alpha]_D^{20} = 66.386 + 0.015035\ p - 0.0003986\ p^2.$$

According to the above equations the maximum specific rotation of sucrose (66.53) is found at $p = 18.86$ per cent; for concentrations lower than this the specific rotation again decreases.

Schmitz † from observations upon eight solutions for $p = 5$ to 65 per cent gives the equation:

$$[\alpha]_D^{20} = 66.510 + 0.004508\ p - 0.00028052\ p^2.$$

Nasini and Villavecchia ‡ for $p = 3$ to 65 give the equation $[\alpha]_D^{20} = 66.438 + 0.010312\ p - 0.00035449\ p^2$. The last named authorities found, however, for very dilute solutions ($c = 0.335$ gm. to 1.2588 gms. sucrose per 100 c.c.) that the specific rotation of sucrose again increases, and for such dilute solutions give the equation $[\alpha]_D^{20} = 69.962 - 4.86958\ p + 1.86415\ p^2$. The variations noted in the above equations for the specific rotation of sucrose are no doubt partly due to the effect of rotation dispersion, as the result of using light of slightly different wave length for illumination.

The equations of Tollens and of Nasini and Villavecchia are considered to be the most accurate. The average of the two equations gives probably the most reliable expression for the specific rotation of sucrose.

I. $[\alpha]_D^{20} = +66.386 + 0.015035\ p - 0.0003986\ p^2$. (Tollens.)

II. $[\alpha]_D^{20} = +66.438 + 0.010312\ p - 0.0003545\ p^2$. (Nasini and Villavecchia.)

Average: III. $[\alpha]_D^{20} = +66.412 + 0.012673\ p - 0.0003766\ p^2$.

* Ber., **10**, 1403. † Ber., **10**, 1414.

‡ Public. de lab. chim. delle gabelle. Rome, 1891, p. 47.

Landolt* by recalculating this combined equation into terms of concentration (grams of sugar per 100 c.c.) gives the expression:

$$\text{IV.}\quad [\alpha]_D^{20} = +66.435 + 0.00870\,c - 0.000235\,c^2 \quad (c = 0 \text{ to } 65).$$

The following table, which with the exception of column *f* is taken from Landolt,* gives a comparison of the specific rotation of sucrose for solutions of different percentage and concentration, according to each of the four preceding equations.

TABLE XXVIII

*Giving Specific Rotation of Sucrose for Different Concentrations*

| *a* | *b* | *c* | *d* | *e* | *f* | *g* |
|---|---|---|---|---|---|---|
| Percentage. | Sp. gr. $\frac{20°}{4°}$ (Tollens.) | Concentration ($c = p.d$). (Tollens.) | Specific rotation $[\alpha]_D^{20}$. By formula I calculated to | By formula II calculated to | By formula III calculated to | By formula IV calculated to |
| $p$ | $d$ | $c$ | $p$ | $p$ | $p$ | $c$ |
| 5 | 1.01786 | 5.0893 | +66.451 | +66.480 | +66.466 | +66.473 |
| 10 | 1.03819 | 10.3819 | 66.496 | 66.506 | 66.501 | 66.500 |
| 15 | 1.05926 | 15.8889 | 66.522 | 66.513 | 66.517 | 66.514 |
| 20 | 1.08109 | 21.6218 | 66.527 | 66.502 | 66.515 | 66.513 |
| 25 | 1.10375 | 27.5938 | 66.513 | 66.474 | 66.493 | 66.496 |
| 30 | 1.12721 | 33.8163 | 66.479 | 66.428 | 66.453 | 66.460 |
| 35 | 1.15153 | 40.3036 | 66.424 | 66.365 | 66.394 | 66.404 |
| 40 | 1.17676 | 47.0704 | 66.350 | 66.283 | 66.316 | 66.324 |
| 45 | 1.20288 | 54.1296 | 66.256 | 66.184 | 66.220 | 66.217 |
| 50 | 1.22995 | 61.4975 | 66.142 | 66.067 | 66.104 | 66.081 |

Concentration equations for the specific rotation of other sugars are given below:

Xylose † ($c = 3$ to 34 gms. per 100 c.c.) $[\alpha]_D^{20} = +18.095 + 0.06986\,p.$

($c = 34$ to 61 gms. per 100 c.c.) $[\alpha]_D^{20} = +23.089 - 0.1827\,p + 0.00312\,p^2.$

Galactose ‡ ($p = 5$ to 35 per cent) $[\alpha]_D^{20} = +79.703 + 0.0785\,p.$

Glucose § ($p = 0$ to 100 per cent) $[\alpha]_D^{20} = +52.50 + 0.018796\,p + 0.00051683\,p^2.$

Fructose || ($p = 1$ to 30 per cent) $[\alpha]_D^{20} = -(91.90 + 0.111\,p).$

Invert sugar ¶ ($p = 9$ to 68 per cent) $[\alpha]_D^{20} = -19.447 - 0.06068\,p + 0.000221\,p^2.$

Maltose ** ($p = 5$ to 35 per cent) $[\alpha]_D^{20} = +138.475 - 0.01837\,p.$

Browne (J. Ind. Eng. Chem., **2,** 526) has calculated the observations of Tollens to concentration and gives the equation for glucose $[\alpha]_D^{20} = +52.50 + 0.0227c + 0.00022\,c^2$.

* "Das optische Drehungsvermögen" (1898), p. 420. † Schulze and Tollens, Ann., **271,** 40. ‡ Meissl, J. prakt. Chem. [2], **22,** 97. § Tollens, Ber., **17,** 2238. || Ost. Ber., **24,** 1636. ¶ Gubbe. Ber., **18,** 2207. ** Meissl, J. prakt. Chem. [2], **25,** 114.

### Effect of Temperature upon Specific Rotation of Sugars

The effect of temperature upon the specific rotation of sugars is no less pronounced than that of concentration and, with a number of sugars such as fructose and galactose, the influence of temperature is the factor which has most to be considered in polarimetric measurements. The change in rotation of a sugar solution due to expansion or concentration in volume through temperature changes must not be confused with changes in specific rotation. In studying the latter phenomenon the sugar solutions must either be made up to volume at the same temperature at which they are to be examined or else a correction be made for the changes in volume due to expansion or contraction.

The influence of temperature upon specific rotation is studied in the same way as that of concentration, by laying off the specific rotation for each temperature upon a diagram. The connecting points for the ordinary ranges of atmospheric temperature lie more nearly in a straight line than is the case with the concentration curves. For wider ranges of temperature, however, the increase or decrease in specific rotation is found to proceed unequally and the change must then be expressed by some curve equation.

**Effect of Temperature upon the Specific Rotation of Sucrose.** — The earlier investigators Mitscherlich, Hesse, and Tuchschmid regarded the effect of temperature upon the specific rotation of sucrose as insignificant. Dubrunfaut* was the first to recognize the fact that increase of temperature caused a decrease in the value of this constant, the temperature coefficient of the specific rotation of sucrose having been found by him to be 0.000232 per 1° C. increase. Andrews,† who reinvestigated the question in 1889, found a decrease of 0.0114 in the specific rotation of sucrose for 1° C. increase. The specific rotation of sucrose for any temperature $t$ is then represented by the equation:

$$[\alpha]_D^t = [\alpha]_D^{20} - 0.0114\ (t - 20).$$

Schönrock‡ in 1896, as a result of observation upon 10 sugar solutions, showed that the decrease in specific rotation for 1° C. increase lay between 0.0132 and 0.0151; for temperatures between 12° C. and 25° C. the change is expressed by the equation:

$$[\alpha]_D^t = [\alpha]_D^{20} - 0.0144\ (t - 20).$$

* Ann. chim. phys. [3], **18**, 201.

† Mass. Inst. Tech. Quarterly, May, 1889, p. 367.

‡ Ber. phys.-techn. Reichsanstalt, 1896.

This equation is sometimes written

$$[\alpha]'_D = [\alpha]^{20}_D - [\alpha]^{20}_D 0.000217\ (t - 20),$$

in which the temperature coefficient of the specific rotation,

$$0.000217 = \frac{0.0144}{[\alpha]^{20}_D} \quad \text{or} \quad \frac{0.0144}{66.5}.$$

Later experiments were made by Schönrock* at temperatures between 9° C. and 32° C. using light of three different wave lengths, the yellow sodium line 589.3 $\mu\mu$, the yellow-green mercury line 546.1 $\mu\mu$, and the blue mercury line 435.9 $\mu\mu$. These experiments showed that for the German normal sugar solution ($p = 23.701$ per cent) the rotation angle underwent a linear deviation with changes in temperature, this deviation being independent of the wave length of light employed. It was found, moreover, that the temperature coefficient of the specific rotation decreased with increase in temperature, the value being 0.000242 at 10° C., 0.000184 at 20° C., and 0.000121 at 30° C. for sodium light. This decrease proceeds in a straight line and the values of the temperature coefficient for any intermediate temperature can be estimated by taking the proportionate difference. These later values of Schönrock are used by the Physikalisch-Technische Reichsanstalt of Germany and have therefore the highest sanction of authority.

**Effect of Temperature upon the Specific Rotation of Other Sugars.** — The effect of temperature upon the specific rotations of a number of other sugars is given in Table XXIX.

TABLE XXIX

| | | |
|---|---|---|
| Rhamnose † | $[\alpha]'_D =$ | $+ 9.18 - 0.035\ t$ ($t = 6°$ to 20° C.) |
| Galactose‡ ($p = 10$) | $[\alpha]'_D =$ | $+ 84.67 - 0.209\ t$ ($t = 10°$ to 30° C.) |
| Fructose§ ($p = 9$) | $[\alpha]'_D =$ | $-103.92 + 0.671\ t$ ($t = 13°$ to 40° C.) |
| Fructose§ ($p = 23.5$) | $[\alpha]'_D =$ | $-107.65 + 0.692\ t$ ($t = 9°$ to 45° C.) |
| Invert sugar‖ ($c = 17.21$) | $[\alpha]'_D =$ | $- 27.9 + 0.32\ t$ ($t = 5°$ to 35° C.) |
| Lactose¶ | $[\alpha]'_D =$ | $+ 52.53 - 0.07\ t - 20$ ($t = 15°$ to 25° C.) |
| Maltose** ($p = 10$) | $[\alpha]'_D =$ | $+140.19 - 0.095\ t$ ($t = 15°$ to 35° C.) |

* Z. Ver. Deut. Zuckerind., **53,** 650.
† Schnelle and Tollens, Ann., **271,** 62.
‡ Meissl, J. prakt. Chem. [2], **22,** 97.
§ Hönig and Jesser, Z. Ver. Deut. Zuckerind., **38** (1888), 1028.
‖ Tuchschmid, J. prakt. Chem. [2], **2,** 235.
¶ Schmöger, Ber., **13,** 1922.
** Meissl, J. prakt. Chem. [2], **25,** 114.

While a linear equation is sufficiently exact for narrow ranges of temperature, the change in specific rotation for wider differences of temperature must usually be expressed by an equation of the order:

$$[\alpha]_D^t = [\alpha]_D^0 + at + bt^2$$

or

$$[\alpha]_D^t = [\alpha]_D^{20} + a(t - 20) + b(t - 20)^2.$$

Gernez,* for example, gives for rhamnose the equation

$$[\alpha]_D^t = 9.22 - 0.03642\,t + 0.0000123\,t^2$$

and Gubbe† gives for invert sugar the following equations:

For $t = 0°$ to 30° C., $[\alpha]_D^t = [\alpha]_D^{20} + 0.3041\,(t - 20) + 0.00165\,(t-20)^2$.

For $t = 20°$ to 100° C., $[\alpha]_D^t = [\alpha]_D^{20} + 0.3246\,(t-20) - 0.00021\,(t-20)^2$.

Sucrose and the different sugars mentioned in Table XXIX all show a decrease in specific rotation with increase in temperature. Of other sugars, which exhibit this property in marked degree, arabinose should be mentioned. Tanret‡ found for l-arabinose $[\alpha]_D^{12} = +105.54$ and $[\alpha]_D^{55} = +88.61$, or an average decrease of 0.394 for 1° C. increase in temperature, which is greater than that for any other sugar except fructose.

Xylose presents an exception to the rule just noted, Schulze and Tollens § having observed for temperatures above 20° C. an increase in specific rotation, as in the following example ($p = 10.0829$).

| $t$ | $[\alpha]_D$ l-xylose. |
|---|---|
| 15° | +18.898 |
| 20 | 18.909 |
| 25 | 19.248 |
| 30 | 19.628 |

Glucose also seems to present an exception to the rule of diminished rotation with increase in temperature. Observations by Dubrunfaut, Mategczek, and others show that the specific rotation of d-glucose undergoes no perceptible change between 0° and 100° C.

Equations giving the combined influence of concentration and temperature upon specific rotation have been worked out for many sugars. The following examples are given:

* Compt. rend., **121,** 1150.
† Ber., **18,** 2207.
‡ Bull. soc. chim. [3], **15,** 195.
§ Ann., **271,** 40.

Galactose* $[\alpha]_D^t = +83.883 + 0.0785\,p - 0.209\,t$. (Meissl.)†

Fructose $[\alpha]_D^t = -[101.38 - 0.56\,t + 0.108\,(c - 10)]$. (Jungfleisch and Grimbert.)‡

Fructose $[\alpha]_D^t = -88.13 - 0.2583\,p + 0.6714\,(t - 20°)$. (Hönig and Jesser.)§

Sorbose $[\alpha]_D^{20} = -[42.65 + 0.047\,p + 0.00007\,p^2 - (t - 20)\,0.02]$. (Tollens and Smith.)‖

Maltose $[\alpha]_D^{20} = +140.375 - 0.01837p - 0.095\,t$. (Meissl.)¶

### Effect of Solvent upon the Specific Rotation of Sugars

The constants of specific rotation for sugars are all expressed for aqueous solutions. It sometimes happens, however, that solutions of sugar in other solvents, such as alcohol, have to be examined; in such cases the changes in specific rotation due to the character of solvent must be taken into account.

In the case of sucrose, Tollens** found the following values for $[\alpha]_D^{20}$ with different solvents for a 10 per cent solution:

| | |
|---|---|
| In water | + 66.667. |
| In 1 part water + 3 parts ethyl alcohol | + 66.827. |
| In 1 part water + 3 parts methyl alcohol | + 68.628. |
| In 1 part water + 3 parts acetone | + 67.396. |

Methyl alcohol and acetone are thus seen to raise the specific rotation of sucrose perceptibly, but ethyl alcohol only slightly. Claassen†† also found for 80 per cent alcohol a slight increase in the specific rotation of sucrose; the differences (0.1 to 0.15), however, are not sufficient to affect seriously the analytical results in such operations as the alcoholic extraction of sugar beet or cane pulp.

In the case of fructose and invert sugar, ethyl alcohol produces a marked lowering of the specific rotation, and when these sugars are present the influence of ethyl alcohol as a solvent must be taken into

* Tanret (Bull. société chimique [3], **15,** 195) gives the change in specific rotation of galactose for 1° C. increase between 13° and 20° −0.39, between 20° and 25° −0.226, and between 25° and 30° −0.180, a falling off in the temperature coefficient with increase in temperature similar to the one noted by Schönrock with sucrose.

† J. prakt. Chem. [2], **22,** 97.

‡ Compt. rend., **107,** 390.

§ Z. Ver. Deut. Zuckerind., **38** (1888), 1028.

‖ Ber., **33,** 1289.

¶ J. prakt. Chem. [2], **25,** 114.

** Ber., **13,** 2287.

†† Z. Ver. Deut. Zuckerind., **40,** 392.

account. Fructose according to Landolt* has a specific rotation in alcohol which is only two-thirds that in water. Borntræger† found for 37.6 gms. invert sugar in 100 c.c. aqueous solution a rotation of −49.2 at 20° C.; when the solution was made up with 10.45 c.c. alcohol the rotation decreased to −43.9 and with 20.60 c.c. alcohol to −38.3. According to Horsin-Deon‡ (whose conclusion, however, requires verification) invert sugar in absolute alcohol is perfectly inactive and only becomes levorotatory upon the addition of water. It should also be noted that the rotation of alcoholic invert-sugar solutions is much more sensitive to changes in temperature than water solutions.

With a number of sugars the specific rotations in aqueous and alcoholic solutions are almost the reverse of one another. The $[\alpha]_D$ of rhamnose§ for example in water is +9.43 and in alcohol −9.0. The $[\alpha]_D$ of sorbose‖ in water is −42.5 and in 85 per cent alcohol +41.8. The effect of pyridine and formic acid upon the specific rotations of several sugars is shown on page 190.

Without giving detailed results of experiments upon all the various sugars it may be said that the effect of solvent upon specific rotation is too great to be disregarded; wherever possible the polarimetric examination of sugars for purpose of analysis should be made in aqueous solution.

### Effect of Accompanying Substances upon Specific Rotation of Sugars

Another factor of importance, especially in the polarimetric examination of impure sugar solutions, is the effect which bases, acids, salts, and other substances exert upon the specific rotation of the sugars present. A very large amount of investigation has been done upon this subject and for complete details reference must be made to the original articles. Only brief mention will be made of the effects of a few substances upon the rotation of the more important sugars.

The changes which foreign optically inactive substances may exert upon the rotation of sugars may be either chemical or physical. The hydroxides of the alkalies and alkaline earths, and all salts of alkaline reaction in general, cause a decrease in the specific rotation of most reducing sugars. Such changes in rotation are purely chemical, being

* Ber., **13,** 2335.
† Z. ang. Chem. (1889), 507.
‡ J. fabr. sucre, **20,** 37.
§ Raýman and Kruis, Bull. soc. chim. [2], **48,** 632.
‖ Adriani, Rec. trav. chim. des Pays Bas., **19,** 184.

due either to a rearrangement of the sugar molecule or to the formation of alkali-sugar compounds of lower specific rotation. The effect of acids and acid salts upon the rotation of sucrose by inversion is another example of purely chemical change. The avoidance of such chemical changes is imperative in accurate polarimetric work and to prevent these the solutions of sugar under examination should be, so far as possible, neutral in reaction.

The influence of neutral salts upon the specific rotation of sugars, on the other hand, is largely physical, since the chemical properties of the dissolved sugars are not appreciably affected; the same is also true of the influence of acids upon the specific rotation of sugars which do not undergo inversion.

**Influence of Mineral Impurities upon the Rotation of Sucrose.** — The chlorides, nitrates, sulphates, phosphates, acetates, and citrates, of the alkalies, the chlorides of the alkaline earths, magnesium sulphate, and many other salts all produce a decrease in the specific rotation of sucrose, this decrease being generally greater with increased amount and smaller molecular weight of salt.

The hydroxides of the alkalies and alkaline earths and the carbonates of the alkalies also lower the specific rotation of sucrose. The influence of these substances, which is of especial importance technically, in view of the alkalinity of various sugar-house products, has been widely studied, the results being often expressed in parts of sugar whose rotation is obscured by one part of alkali. Pellet for example gives the following results:

| Substance. | Concentration of sucrose solution. | |
|---|---|---|
| | 5.4 gms. 100 c.c. | 17.3 gms. 100 c.c. |
| | Grams sucrose. | Grams sucrose. |
| 1 gm. caustic potash obscures rotation of | 0.170 | 0.500 |
| 1 gm. caustic soda obscures rotation of | 0.140 | 0.450 |
| 1 gm. potassium carbonate obscures rotation of | 0.044 | 0.065 |
| 1 gm. sodium carbonate obscures rotation of | 0.040 | 0.132 |
| 1 gm. calcium oxide obscures rotation of | 0.7 | 1.0 |
| 1 gm. barium oxide obscures rotation of | 0.190 | 0.430 |

Strontium oxide also diminishes the specific rotation of sucrose. This lowering effect of alkalies upon the specific rotation of sucrose is largely due to the formation of soluble saccharates of lower specific rotation; the influence can be largely eliminated by neutralization with acetic acid. The original specific rotation is not entirely restored,

however, since the soluble acetates themselves lower the specific rotation of sucrose to a slight extent.

The probable effect of a mixture of salts upon the polarization of sucrose, — such for example as occurs in beet molasses, which contains about 50 per cent of sucrose and 10 per cent of soluble salts (mostly of potassium), — may be judged from the following examples taken from experiments by Bodenbender and Steffens.*

TABLE XXX

| Salt. | Sucrose, parts. | Salt, parts. | Water, parts. | Polarization, sugar degrees. | Difference. |
|---|---|---|---|---|---|
| Potassium chloride | 5 | 1 | 94 | 4.987 | 0.013 |
| | 10 | 2 | 88 | 9.856 | 0.144 |
| | 20 | 4 | 76 | 19.869 | 0.131 |
| Sodium chloride | 5 | 1 | 94 | 4.969 | 0.031 |
| | 10 | 2 | 88 | 9.853 | 0.147 |
| | 20 | 4 | 76 | 19.586 | 0.414 |
| Barium chloride | 5 | 1 | 94 | 4.952 | 0.048 |
| | 10 | 2 | 88 | 9.944 | 0.056 |
| | 20 | 4 | 76 | 19.402 | 0.598 |
| Magnesium sulphate | 5 | 1 | 94 | 4.995 | 0.005 |
| | 10 | 2 | 88 | 9.890 | 0.110 |
| | 20 | 4 | 76 | 19.880 | 0.120 |
| Sodium phosphate | 5 | 1 | 94 | 4.958 | 0.042 |
| | 10 | 2 | 88 | 9.933 | 0.067 |
| | 20 | 4 | 76 | 19.689 | 0.311 |
| Potassium carbonate | 5 | 1 | 94 | 4.927 | 0.073 |
| | 10 | 2 | 88 | 9.730 | 0.270 |
| | 20 | 4 | 76 | 19.300 | 0.700 |
| Sodium carbonate | 5 | 1 | 94 | 4.910 | 0.090 |
| | 10 | 2 | 88 | 9.711 | 0.289 |
| | 20 | 4 | 76 | 19.173 | 0.827 |

The effect of four-fold concentration is seen to depress the difference in rotation about ten times, so that an apparent loss of sucrose may seem to take place in the evaporation of sugar solutions rich in mineral salts, when such solutions are examined by the polariscope before and after evaporation.

The effect which the various salts, used for clarifying impure sugar solutions for optical analysis, exercise upon the specific rotation of sucrose and other sugars is also of great importance. Lead subacetate is the salt most used for this purpose; its effect upon the rotation of sucrose is considered elsewhere (page 216).

**Influence of Mineral Impurities upon the Rotation of Reducing Sugars.** — The action of salts of alkaline reaction in depressing the rotation of reducing sugars has already been mentioned. In sacchari-

* Z. Ver. Deut. Zuckerind., **31**, 808.

metric analysis the influence of lead subacetate, as a clarifying agent, upon the rotations of fructose and invert sugar, is of great importance. As was first observed by Gill* in 1871 when solutions containing invert sugar are treated with lead-subacetate solution in excess, the formation of soluble lead fructosate of low specific rotation is so pronounced that the rotatory power of fructose sinks below that of glucose and the invert sugar becomes dextrorotatory. Similar observations have been made by Pellet, Bittmann, Koydl, Borntraeger, and many others. In the following experiments by Bittmann† 50 c.c. of invert-sugar solution were treated with 50 c.c. of a mixture of water and lead subacetate in different proportions.

| Water. | Lead-subacetate solution. | Polarization. |
|---|---|---|
| c.c. | c.c. | |
| 50 | 0 | −2.3 |
| 40 | 10 | −1.0 |
| 30 | 20 | +3.7 |
| 10 | 40 | +7.5 |

The influence of neutral salts upon the specific rotation of reducing sugars is variable. Some salts produce an increase, others a decrease and some no change whatever in rotation; no general rule can be given.

Of particular importance in this connection is the influence of different neutral salts upon the rotation of invert sugar; the occurrence of such salts in molasses and other low-grade sugar-house products may increase the levorotation of the invert sugar several degrees, with the result that erroneous conclusions are sometimes drawn from the polariscopic examination of such products.

**Influence of Acids upon the Specific Rotation of Sugars.** — The presence of free mineral acids exerts a very pronounced influence upon the specific rotation of certain sugars. This influence is very slight in case of glucose, but is most pronounced with fructose and hence also with invert sugar. O'Sullivan, for example, found for invert sugar, prepared by inverting sucrose with invertase, $[\alpha]_D^{15} = -24.5$, and for invert sugar, prepared by inverting sucrose with sulphuric acid in the cold, $[\alpha]_D^{15} = -27.7$, an increase of 3.2, which if referred entirely to fructose would mean an increase of 6.4 in the specific rotation of that sugar. The increase in rotation increases with the amount of acid, as is seen from the following results by Hammerschmidt‡ which the author

* Z. Ver. Deut. Zuckerind., **21,** 257.
† Ibid., **30,** 875.
‡ Ibid., **40,** 465; **41,** 157.

has calculated to the $[\alpha]_D^{20}$ of invert sugar and fructose. The results were obtained by inverting a half-normal weight of sucrose with varying amounts of concentrated hydrochloric acid and then completing the volume to 100 c.c.

TABLE XXXI

*Showing Influence of Varying Quantities of Hydrochloric Acid upon the Rotation of Invert Sugar and Fructose.*

| Volume of HCl added. | Observed saccharimeter reading, 20° C. (13.6842 gms. invert sugar to 100 c.c.) | Calculated $[\alpha]_D^{20}$ | |
|---|---|---|---|
| | | Invert sugar. | Fructose. |
| c.c. | °V. | | |
| 0 | ..... | −20.00 | −92.50 |
| 5 | −16.50 | −20.89 | −94.28 |
| 10 | −17.06 | −21.60 | −95.70 |
| 15 | −17.58 | −22.26 | −97.02 |
| 20 | −18.02 | −22.82 | −98.14 |

The influence of the change in specific rotation of fructose upon the determination of sucrose by the methods of acid inversion is discussed on page 270. The action of organic acids upon the rotation of fructose and invert sugar is much less pronounced than that of mineral acids, and can usually be disregarded in polariscopic analysis.

**Influence of Foreign Optically Active Substances upon the Specific Rotation of Sugars.** — The effect of other optically active ingredients upon the rotation of a sugar is of importance especially in determining the polarizing power of several sugars in solution or of mixtures of sugars with organic non-sugars which are optically active. The difficulties in conducting studies of this kind seem to have deterred investigation somewhat; the results upon the polarizing power of sugar mixtures, so far as they have been carried, show, however, no change in the rotation of the individual sugars.

The polarizing power, for example, of solutions of sucrose and glucose in different proportions was found by Hammerschmidt* to agree with the sum of the values calculated by the concentration formulæ of Tollens (page 177) within experimental limits of error. Similar results were also obtained by Creydt† in case of cane sugar and raffinose. Results by Browne‡ upon the polarization of mixtures of glucose and fructose, glucose and galactose, fructose and galactose, fructose and

* "Das specifishe Drehungsvermögen von Gemengen optisch activer Substanzen," Dissertation, Rostock University, 1889.

† Z. Ver. Deut. Zuckerind. (1887), **37**, 153.

‡ J. Am. Chem. Soc., **28** (1906), 339.

arabinose, arabinose and xylose also show that it is safe to assume in analytical work that the specific rotation of these sugars is not perceptibly affected by other sugars in solution.

## Mutarotation

A phenomenon observed in the polarization of all optically active reducing sugars is that of mutarotation (also called birotation or multirotation). The polarizing power of such sugars undergoes after solution at first a rapid change which slowly becomes more gradual until after a few hours the polariscope reading remains constant. This phenomenon was first observed upon glucose in 1846, by Dubrunfaut* and the fact that the initial rotation of this sugar was about twice the constant value caused the introduction of the name birotation. The relation 2 : 1 was found, however, to be different in the case of other sugars; Wheeler and Tollens,† for example, found the ratio in case of xylose to be about 4.5:1 and accordingly suggested the name multirotation. This term, however, in recent years has given place to the more expressive word mutarotation (Latin *mutare* = to change) introduced by Lowry‡ in 1899.

The effect of mutarotation upon the rotatory power of sugars is shown in the following table, in which results are quoted from the work of Tollens and his coworkers, giving the specific rotation of a number of sugars directly after solution and after standing until no further change was noted. The time after solution is given after each value for $[\alpha]_D^{20}$.

Table XXXII

*Showing Mutarotation of Different Sugars*

| Sugar. | Grams per 100 c.c. | $[\alpha]_D^{20}$ initial. | | $[\alpha]_D^{20}$ constant. | | Difference. | Velocity constant (Osaka). |
|---|---|---|---|---|---|---|---|
| | | | min. | | hours | | |
| l-Arabinose..... | 9.73 | +156.7 | 6.5 | +104.6 | 1.5 | −52.1 | 0.031 |
| l-Xylose..... | 10.235 | + 85.9 | 5. | + 18.6 | 2.0 | −67.3 | 0.022 |
| d-Glucose..... | 9.097 | +105.2 | 5.5 | + 52.5 | 4.5 | −52.7 | 0.0104 |
| d-Galactose.... | 10.000 | +117.4 | 7. | + 80.3 | 4.5 | −37.1 | 0.0102 |
| d-Fructose..... | 10.000 | −104.0 | 6. | − 92.3 | 0.5 | −11.7 | 0.096 |
| Rhamnose..... | 10.000 | − 5.0 | 5.5 | + 9.4 | 1.0 | +14.4 | 0.039 |
| Fucose..... | 6.916 | −111.8 | 11. | − 77.0 | 2.0 | −34.8 | 0.022 |
| Lactose..... | 4.841 | + 87.3 | 8. | + 55.3 | 10.0 | −32.0 | 0.0046 |
| Maltose..... | 9.2 | +118.8 | 6. | +136.8 | 6.5 | +17.0 | 0.0072 |

* Compt. rend., **23,** 38.

† Ann., **254,** 312.

‡ J. Chem., Soc., **75,** 212.

It is noted that in case of rhamnose there is a decrease in rotation from − 5.0 to 0 and then an increase from 0 to + 9.4. Maltose also differs from the other sugars in showing a less rotation at time of solution than after standing.

**Effect of Temperature on Mutarotation.** — The speed of mutarotation is influenced by a large number of factors. It is accelerated by increase in temperature, the change proceeding very slowly at 0° C., and almost instantly at 100° C. Dilute sugar solutions show the same velocity of change for all concentrations. Highly concentrated solutions, however, do not always give the true end rotation; such solutions must first be diluted and then allowed to stand for the change in rotation to be completed. This fact must be borne in mind in the polariscopic examination of concentrated sugar solutions, such, for example, as liquid honey, otherwise a considerable error may be introduced in the work of analysis.

**Velocity of Mutarotation.** — The velocity of the change from initial to constant rotation is different for different sugars, and also varies according to temperature, solvent, and other conditions. Urech * was the first to show that the speed of mutarotation followed the same law as that noted by Wilhelmy in the inversion of sucrose (page 660), and which is expressed by the following general formula for a reaction of the first order,

$$\frac{dx}{dt} = k\,(a - x),$$

in which $k$ is the coefficient of velocity, $a$ the total change between the beginning and end point, and $x$ the change at the end of any time $t$. The above equation by integration gives

$$k = \frac{1}{t} \log \frac{a}{a - x}.$$

Owing to the impossibility of measuring the specific rotation of a sugar at the exact moment of solution, the velocity of mutarotation is generally determined by the modified formula

$$k = \frac{1}{t_2 - t_1} \log \left(\frac{\beta_1 - \phi}{\beta_2 - \phi}\right),$$

in which $\beta_1$ and $\beta_2$ are the rotations at the end of the corresponding times $t_1$ and $t_2$, and $\phi$ the constant end rotation.

The method of calculation is shown by the following example, which is taken from the work of Levy.†

* Ber., **16,** 2270; **17,** 1547; **18,** 3059.

† Z. physik. Chem., **17,** 301.

TABLE XXXIII

*Showing Velocity of Mutarotation for a Glucose Solution*

Per cent, $C_6H_{12}O_6 = 3.502$. $d\frac{20°}{4°} = 1.0114$. Temperature = 20.5° to 20.9° C.

| Time after solution. | Angular rotation (8 *dm.* tube). | $t_2 - t_1$ | Temperature. | $k = \frac{1}{t_2 - t_1} \log_{10}\left(\frac{\beta_1 - \phi}{\beta_2 - \phi}\right)$ |
|---|---|---|---|---|
| $t_1 = 25$ min. | $\beta_1 = 27.865°$ | 0 | 20.9° C. | ..... |
| $t_2 = 30$ min. | $\beta_2 = 27.060$ | 5 | 20.9 | 0.00649 |
| $t_2 = 35$ min. | $\beta_2 = 26.159$ | 10 | 20.9 | 0.00719 |
| $t_2 = 40$ min. | $\beta_2 = 25.637$ | 15 | 20.8 | 0.00644 |
| $t_2 = 45$ min. | $\beta_2 = 24.927$ | 20 | 20.7 | 0.00662 |
| $t_2 = 50$ min. | $\beta_2 = 24.369$ | 25 | 20.6 | 0.00652 |
| $t_2 = 55$ min. | $\beta_2 = 23.895$ | 30 | 20.5 | 0.00636 |
| $t_2 = 60$ min. | $\beta_2 = 23.166$ | 35 | 20.5 | 0.00677 |
| $t_2 = 65$ min. | $\beta_2 = 22.797$ | 40 | 20.5 | 0.00656 |
| $t_2 = 70$ min. | $\beta_2 = 22.171$ | 45 | 20.5 | 0.00687 |
| $t_2 = 75$ min. | $\beta_2 = 21.837$ | 50 | 20.5 | 0.00674 |
| $t_2 = 80$ min. | $\beta_2 = 21.470$ | 55 | 20.5 | 0.00671 |
| $t_2 = 85$ min. | $\beta_2 = 21.088$ | 60 | 20.5 | 0.00675 |
| 24 hours | $\phi = 16.692$ | ..... | Average | 0.00662 |

The velocity constants by Osaka* given in Table XXXII were calculated by this method. It is seen that the change to constant rotation is most rapid for fructose and slowest for lactose.

**Effect of Acids, Bases, and Salts on Mutarotation.** — The action of acids, bases, and salts upon the velocity of mutarotation has been a subject of considerable study. Acids accelerate the change according to their degree of dissociation, or electric conductivity, preserving approximately the same order as that noted in the inversion of sucrose. Levy,† for example, gives the following constants for the speed of mutarotation of glucose in presence of different acids ($\frac{1}{10}$ normal) and the relative acceleration of each acid in terms of hydrochloric acid = 100.

TABLE XXXIV

*Showing Acceleration of Different Acids upon Mutarotation*

| In presence of. | Velocity constant of mutarotation. | Temperature. | Relative acceleration. |
|---|---|---|---|
| Water..... | 0.00610 | 20.1° C. | |
| Water..... | 0.00637 | 20.25 | |
| ..... | ..... | ..... | ..... |
| Hydrochloric acid..... | 0.02300 | 20.25 | 100.00 |
| Nitric acid..... | 0.02283 | 20.1 | 98.99 |
| Trichloracetic acid..... | 0.02325 | 20.25 | 96.67 |
| Sulphuric acid..... | 0.01886 | 20.0 | 71.95 |
| Dichloracetic acid..... | 0.01670 | 20.2 | 62.41 |
| Monochloracetic acid .. | 0.01004 | 20.25 | 17.25 |
| Acetic acid..... | 0.00716 | 20.2 | 4.70 |
| Propionic acid..... | 0.00636 | 19.8 | 1.63 |

* Z. physik. Chem., **35**, 661. † Z. physik. Chem., **17**, 301.

The values for relative acceleration of the different acids preserve the same order as those noted for the inversion constants in Table XCV (page 663).

It is scarcely necessary to state that the speed of mutarotation increases with the strength of acid employed. Thus Levy found for $n/10$ hydrochloric acid, $k = 0.02300$ and for $n/50$ hydrochloric acid, $k = 0.00971$; for $n/10$ acetic acid, $k = 0.00716$ and for $n/50$ acetic acid, $k = 0.00654$.

Alkalies also accelerate the speed of mutarotation, the change to constant rotation being almost instantaneous. Schulze and Tollens* using 0.1 per cent ammonia obtained the normal constant rotation with arabinose, xylose, rhamnose, galactose, glucose, fructose, and lactose within 9 minutes; $n/200$ alkali (KOH) gives the end rotation of glucose almost instantly. The use of much stronger alkali, however, induces chemical change with a decrease of the rotation below the normal value. Trey† for example using 0.2 gm. sodium hydroxide per 100 c.c. obtained as the $[\alpha]_D$ for glucose after 15 minutes + 52.7 (normal), after 24 hours + 36.7, after 48 hours + 26.0, after 34 days + 15.1, and after 65 days − 0.4.

The different salts nearly all accelerate the speed of mutarotation, those of alkaline reaction standing first in this respect. Sodium chloride, however, presents an exception to this rule, having been found by Levy‡ and also by Trey§ to cause the mutarotation of glucose to proceed slower than in pure aqueous solution.

Mutarotation of sugars takes place not only in water but also in other solvents such as absolute methyl alcohol, ethyl alcohol, acetone, etc. The change in rotation proceeds much more slowly, however, in organic solvents than in aqueous solution. This is shown in the following results by Grossmann and Bloch‖ which give the mutarotation of several sugars in pyridine and formic acid.

| Sugar. | $[\alpha]_D$ in pyridine. | | | | $[\alpha]_D$ in formic acid. | | | |
|---|---|---|---|---|---|---|---|---|
| | After solution. | | Constant. | | After solution. | | Constant. | |
| | | Min. | | Days. | | Min. | | Days. |
| Xylose..... | +117.39 | 8 | + 40.63 | 4 | + 40.34 | 4 | + 66.60 | 2 |
| Rhamnose.... | − 41.39 | 5 | − 32.77 | 4 | + 10.20 | 5 | − 35.76 | 6 |
| Galactose.... | +154.28 | 23 | + 59.83 | 3 | + 89.11 | 5 | +127.35 | 5 |
| Glucose..... | +149.60 | 10 | + 74.79 | 4 | + 72.16 | 5 | +122.51 | 4 |
| Fructose..... | −174.13 | 10 | − 34.83 | 1 | − 94.32 | 5 | −47.83 | 8 |
| Maltose..... | +103.48 | 15 | +123.80 | 11 | +129.11 | 10 | +172.15 | 3 |

* Ann., **271,** 49. † Z. physik. Chem., **22,** 439. ‡ Ibid., **17,** 320.
§ Z. physik. Chem., **22,** 424. ‖ Z. Ver. Deut. Zuckerind., **62,** 19.

A peculiarity of xylose and rhamnose in pyridine is an increase in the rotation after solution. Grossmann and Bloch observed a maximum of + 122.07 in case of xylose 15 minutes after solution and a maximum of − 45.92 in case of rhamnose 30 minutes after solution. It is seen that mutarotation in the two solvents proceeds in many cases in opposite directions and that there is no relation between the constant rotations and those observed in aqueous solution. The addition of water to solutions of sugar in organic solvents accelerates, and conversely the addition of alcohol, acetone, etc., to aqueous solutions retards, the speed of mutarotation. As a general rule the presence of any soluble non-electrolyte, such, for example, as sucrose, will increase the time necessary for a mutarotating sugar to reach constant polarization.

Mutarotation takes place not only after dissolving reducing sugars, but also occurs upon the liberation of these sugars from higher saccharides by the action of enzymes. The phenomenon is one which the sugar chemist has always to bear in mind. Polariscopic measurements are always referred to the normal constant rotation. The latter condition may be produced almost instantly by heating the solution or by adding a little free alkali, but when such means are employed care must be taken to prevent the liability of chemical change. The safest course is to allow the solution to stand until the rotation has come to equilibrium in the natural way.

**Theories of Mutarotation.** — Many theories have been proposed to explain mutarotation. According to the views of Landolt* and other authorities it was thought that the phenomenon might be due to the formation of molecular aggregates immediately after solution, which afterwards decompose into simple molecules of lower rotation. These earlier theories were largely disproved, however, by the experiments of Arrhenius,† and of Brown and Morris,‡ who showed that no change occurred in the molecular weight of a sugar during mutarotation. Tollens§ and others of his school have supposed that mutarotation might be caused by the formation of unstable hydrates which, by the splitting off of water, cause a change in rotation.

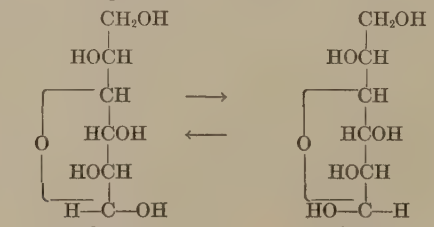
Much additional light was thrown upon the subject in 1895 by Tanret,‖ who discovered that sugars could exist in both a high- and a low-mutarotating form. The relationship of these several modifications, according to Tanret's classification, is shown for four different sugars in the following table.

* "Das optische Drehungsvermögen" (1879), 58.
† Z. physik. Chem., **2**, 500.
‡ Chem. News, **57**, 196.
§ Ber., **26**, 1799.
‖ Compt. rend., **120**, 1060.

| Sugar. | $\alpha$ Metastable. | $\beta$ Stable. | $\gamma$ Metastable. |
|---|---|---|---|
| d-Glucose | +105° | +52.5° | +22.5° |
| d-Galactose | +135 | +81 | +52 |
| Lactose | + 88 | +55 | +36 |
| Rhamnose | − 6 | + 9 | +23 |

Tanret's $\alpha$ modification represents the ordinary sugar as obtained by crystallization from aqueous solution. The $\beta$ modification, or form of constant rotation, was usually obtained by precipitating a saturated aqueous solution of the $\alpha$ sugar with several volumes of absolute alcohol. The $\gamma$ modification was usually prepared by evaporating a concentrated solution of the $\alpha$ sugar to dryness and then heating for several hours to about 100° C. Repeating the process several times increases the purity of the various modifications. In the case of rhamnose the $\alpha$ modification is the lower, and the $\gamma$ modification the higher rotating form.

Previous to Tanret's work, Lippmann* had expressed the view that mutarotation might be due to a stereochemical change between two forms of the same sugar, and showed, how by adopting a form of structure first proposed by Tollens, that one of the terminal carbon atoms of the sugar molecule became asymmetric (i.e. connected to four dissimilar atoms or groups), thus permitting the existence of two configurations for the same sugar. The theory of mutarotation most generally accepted at the present time assigns one of these configurations to the high-rotating, and the other configuration to the low-rotating form. The mutarotation reaction according to Lowry† is thus regarded as a balanced reaction between two molecular forms of the same sugar, as for example:




$\alpha$ glucose $[\alpha]_D = +105$ $\beta$ glucose $[\alpha]_D = +22.5$.

Which of the above configurations belongs to the $\alpha$ or $\beta$ sugar has not been determined.

* Ber., **29**, 203. † J. Chem. Soc., **75**, 212.

Lowry's view was supported by Hudson,* who showed by quantitative experiments that the change between the high- and low-rotating forms of lactose was a balanced reaction. According to this view, Tanret's solid $\beta$ sugars of constant rotation are simply equilibrated mixtures of the high- and low-rotating forms. The designation $\beta$ is applied at present to Tanret's $\gamma$ modification.

While mutarotation is most generally regarded at present as a balanced reaction between high- and low-rotating forms, the intermediate steps of the process have not been definitely established. The change in polarization of a sugar solution to constant rotation is regarded by some chemists as simply a conversion of the $\alpha$ or $\beta$ oxygen ring compound into the ordinary aldehyde or ketone form. Other chemists regard the solution at constant rotation as containing simply a mixture of the $\alpha$ and $\beta$ sugars in equilibrium, while still others believe it to contain the $\alpha$ and $\beta$ sugars with variable amounts of the aldehyde or ketone form. For a review of the different hypotheses, which have been proposed in this connection, the chemist is referred to the various special works.†

* Z. physik. Chem., **44,** 487. See also page 711.

† Lippmann, "Chemie der Zuckerarten" (1904), 293.

Hudson (J. Am. Chem. Soc., **32,** 889) in a paper entitled "A Review of Discoveries on the Mutarotation of Sugars," gives a very complete review and bibliography of the subject.

# CHAPTER IX

## METHODS OF SIMPLE POLARIZATION

### Determination of Sugars from Angular Rotation

The amount of a single optically active sugar, in presence of optically inactive substances or in presence of substances without effect upon its specific rotation, may be calculated by means of either formula for specific rotation (page 172).

$$[\alpha]_D = \frac{100\,a}{l \times c} \quad \text{whence} \quad c = \frac{100\,a}{l \times [\alpha]_D}. \tag{1}$$

$$[\alpha]_D = \frac{100\,a}{l \times p \times d} \quad \text{whence} \quad p = \frac{100\,a}{l \times d \times [\alpha]_D}. \tag{2}$$

As to which of the above methods of calculation is to be used, the first or concentration formula is the better where a definite weight of substance is made up to volume before polarization, the usual method of procedure; in case, however, a sugar solution of known specific gravity is polarized directly, then the second or percentage formula is to be employed.

The following formulæ are given for calculating the concentration (grams per 100 c.c.) of different sugars from the angular rotation ($a$) in a 2-dm. tube.

1. Arabinose $c = \dfrac{100\,a}{2 \times +104.5} = 0.4785\,a.$
2. Xylose $c = \dfrac{100\,a}{2 \times +19.0} = 2.6316\,a.$
3. Glucose $c = \dfrac{100\,a}{2 \times +52.8} = 0.9470\,a.$
4. Fructose $c = \dfrac{100\,a}{2 \times -92.5} = 0.5405\,a$ (left degrees).
5. Galactose $c = \dfrac{100\,a}{2 \times +81.0} = 0.6173\,a.$
6. Sucrose $c = \dfrac{100\,a}{2 \times +66.5} = 0.7519\,a.$
7. Maltose $c = \dfrac{100\,a}{2 \times +138.0} = 0.3623\,a.$

8. Lactose $c = \frac{100\,a}{2 \times + 52.5} = 0.9524\,a.$

9. Raffinose $(+ 5\ H_2O)$ $c = \frac{100\,a}{2 \times + 104.5} = 0.4785\,a.$

10. Raffinose (anhydride) $c = \frac{100\,a}{2 \times + 123.15} = 0.4060\,a.$

The percentage $p$ of a sugar in solution is equal to the value of $c$, as expressed above, divided by the specific gravity of the solution.

Such formulæ, as the above, are sufficiently accurate for most purposes of analysis. In cases, however, where the specific rotation of the sugar is affected by changes in concentration or temperature, the results as obtained above can be considered only approximate; to obtain the correct concentration or percentage, it is necessary to calculate the specific rotation corresponding to the approximate value of $c$ or $p$ at the temperature of polarization and substitute this corrected specific rotation in formulæ (1) or (2) for the final calculation of $c$ or $p$.

*Example.* — 50 gms. of a dextrose sirup were dissolved to 100 cc.; the constant rotation of the solution thus obtained was + 34.55 circular degrees in the 200-mm. tube. Required the percentage of dextrose in the sirup.

From formula 3 we obtain by substitution $c = 0.9470 \times 34.55 = 32.72$ gms. dextrose in the 100 cc. of solution or for the 50 gms. of sirup, 65.44 per cent approximately. The specific rotation of dextrose for $c = 32.72$ is found from the formula $[\alpha]_D^{20^\circ} = + 52.50 + 0.0227\,c + 0.00022\,c^2$ (p. 177) to be + 53.48; substituting this in the general formula for $c$ we obtain

$$c = \frac{100 \times 34.55}{2 \times 53.48} = 32.30 \text{ gms. dextrose}$$

in the 100 cc. of solution or for the 50 gms. of sirup the true percentage 64.60, — 0.84 per cent less than the value by the uncorrected formula.

By modifying the formula for $c$, so as to correct for the variations in specific rotation, the labor of the second calculation in the above example may be eliminated. In the case of glucose, by calculating the angular rotation, $(a)$ for the 2-dm. tube, corresponding to concentrations ranging from 10 to 60, we obtain, using the method of least squares (p. 165), the formula $c^* = 0.958\,a - 0.00067\,a^2$.

*Example.* — Applying the last formula to the previous example, we obtain for $c$, 32.299 gms. dextrose in the 100 cc. of solution or for the 50 gms. sirup 64.60 per cent.

* For $p$ Landolt gives the formula $p = 0.948\,a - 0.0032\,a^2$. ("Optisches Drehungsvermögen," p. 447.)

## Determination of Sugars from Saccharimeter Readings

**Conversion of Saccharimeter Readings into Angular Rotation.**—The general methods of optical analysis just described are more especially applicable to polarimeters, where readings are taken in angular degrees; the formulæ given are equally applicable, however, to saccharimeters in which case the scale reading of the latter must be converted into angular degrees by means of the proper conversion factor. For general purposes the factor established for sucrose may be applied to other sugars. In the case of the Ventzke scale, sugar degrees $\times$ 0.34657 = angular rotation. Since, however, the rotation dispersion of the various sugars, with reference to the quartz compensation of the saccharimeter, may differ somewhat from that of sucrose, it is always better, where exact data are available (which is unfortunately not always the case), to use the conversion factor established for the particular sugar. In the case of a few sugars Landolt* has established the following factors for converting divisions of the Ventzke scale into circular degrees.

| | |
|---|---|
| Sucrose | 0.3465 |
| Lactose | 0.3452 |
| Glucose | 0.3448 |
| Invert sugar | 0.3432 |
| Raffinose | 0.3450 |

Brown, Morris, and Millar† give the following:

| | |
|---|---|
| Sucrose, 10 per cent solution | 0.3469 |
| Maltose, 10 per cent solution | 0.3449 |
| Maltose, 5 per cent solution | 0.3457 |
| Glucose, 10 per cent solution | 0.3442 |
| Glucose, 5 per cent solution | 0.3454 |
| Starch products, 10 per cent solution | 0.3458 |
| Starch products, 5 per cent solution | 0.3454 |

Herzfeld,‡ with a solution containing 11.29 per cent anhydrous maltose, obtained upon a Peters saccharimeter, using a Welsbach light with chromate filter, a reading of 93.88 Ventzke degrees at 20° C., and with the same solution upon a Lippich polarimeter a reading of 32.60 circular degrees at 20° C. The value of a Ventzke-scale division for maltose under these conditions is therefore $\frac{32.60}{93.88} = 0.3471$ circular degree, a figure perceptibly greater than the values of Brown, Morris, and Millar. Differences in concentration of the sugar solutions examined but more especially differences in the optical center of gravity of the light employed for illuminating the saccharimeter are the chief

* Ber., **21**, 194. † J. Chem. Soc. Trans., **71**, 92. ‡ Ber., **28**, 441.

causes of such discrepancies. The chemist should, therefore, employ any prescribed conversion factor with caution and use it only under the same conditions for which it was established. It is also well to verify a conversion factor wherever possible, by comparative readings of the same sugar solution upon a polarimeter. The latter instrument does away with the errors of rotation dispersion and, aside from the objection of using monochromatic light, is always to be preferred in methods where the concentration or percentage of sugar is calculated from the angular rotation. If a quartz-wedge saccharimeter is the only instrument available, the average factor 0.346 may be used for most purposes without serious error.

**Normal Weights of Sugars.** — If a normal weight of each particular sugar be taken for polarization, (i.e. the weight of pure sugar which dissolved to 100 c.c. will give a scale reading of 100), the percentage (uncorrected) of sugar may be read directly upon the saccharimeter.

There are a number of methods of calculating the normal weight for different sugars. If we assume in case of the Ventzke scale that the angular rotation of each division is 0.34657 circular degree for all sugars, then the normal weight (20° C., 100 true c.c.) of any sugar, for the 2-dm. observation tube, as compared with 26.00 gms., will be inversely proportional to the specific rotations of this sugar and of sucrose, that is:

$[\alpha]_D^{20}$: 66.5 : : 26 gms. : $X$, whence $X$ (the normal weight) $= \frac{1729}{[\alpha]_D^{20}}$.

The normal weights of several sugars calculated by this method are given in the following table:

TABLE XXXV

*Giving Normal Weights of Different Sugars for Ventzke Scale*

| Sugar. | Specific rotation $[\alpha]_D^{20}$. | Normal weight. |
|---|---|---|
| Glucose | +53.46 $c$=32.5 gms. | $\frac{1729}{53.46}$=32.342 gms. |
| Fructose | −93.00 $c$=18.5 gms. | $\frac{1729}{93}$=18.592 gms. |
| Invert sugar | −20.00 $c$=10.0 gms. | $\frac{1729}{20}$=86.450 gms. |
| Lactose (+$H_2O$) | +52.53 | $\frac{1729}{52.53}$=32.914 gms. |
| Maltose | +138.25 $c$=12.5 gms. | $\frac{1729}{138.25}$=12.506 gms. |
| Raffinose (+5 $H_2O$) | +104.5 | $\frac{1729}{104.5}$=16.545 gms. |
| Raffinose (anhydride) | +123.17 | $\frac{1729}{123.17}$=14.037 gms. |

While the normal weights calculated in this manner are sufficiently exact for most purposes of analysis they must not be regarded as absolute. Owing to the differences, previously mentioned, in rotation dispersion for the different sugars the angular rotation of each Ventzke-scale division will vary slightly from 0.34657 circular degree with a corresponding change in the value of the normal weight.

If the value of the 100-degree saccharimetric reading of each sugar has been established in circular degrees, for the same conditions under which analyses are made, it is always better to base the calculation of the normal weight upon this. The method of calculation for the Ventzke scale, using as illustrations four of the sugars previously taken, is as follows:

From the general formula $c = \frac{100\,a}{l \times [\alpha]_D}$ we obtain for

Glucose

(1° V. = 0.3448 circular degree, Landolt), $c = \frac{100 \times 34.48}{2 \times 53.46} = 32.248$ gms.

Lactose

(1° V. = 0.3452 circular degree, Landolt), $c = \frac{100 \times 34.52}{2 \times 52.53} = 32.857$ gms.

Maltose

(1° V. = 0.3449 circular degrees, Brown, Morris, and Millar) $c = \frac{100 \times 34.49}{2 \times 138.25} = 12.474$ gms.

Raffinose + 5 $H_2O$

(1° V. = 0.3450 circular degree, Landolt), $c = \frac{100 \times 34.50}{2 \times 104.5} = 16.507$ gms.

The conversion factors to be employed, and hence the values of the normal weights, will necessarily depend upon the quality of the light used for illuminating the saccharimeter. The value of a saccharimeter division in circular degrees for a solution of the sugar of the approximate concentration, should, therefore, be established by the chemist himself wherever possible.

*Correction for Concentration and Temperature.* — When normal weights of the different sugars are used, the observed saccharimeter readings require correction for changes in concentration and temperature as described on page 195. Where much work is done with a single sugar a table of corrections should be prepared, giving the actual sugar value corresponding to each scale division of the saccharimeter. The correction table for sucrose (page 118) or the following results calculated by Browne* for glucose upon the basis of the normal weight of 32.25 gms. will illustrate the method.

* J. Ind. Eng. Chem., **2**, 526.

| Scale division. | Concentration. Grams glucose 100 true c.c. 20° C. | Specific rotation, glucose $[\alpha]_D^{20}$. | Actual glucose value of scale division. | Correction to be added. |
|---|---|---|---|---|
| 100° V. | 32.250 | 53.46 | 100.00 | 0.00 |
| 90 | 29.025 | 53.34 | 90.20 | 0.20 |
| 80 | 25.800 | 53.23 | 80.35 | 0.35 |
| 70 | 22.575 | 53.12 | 70.45 | 0.45 |
| 60 | 19.350 | 53.02 | 60.50 | 0.50 |
| 50 | 16.125 | 52.92 | 50.51 | 0.51 |
| 40 | 12.900 | 52.83 | 40.48 | 0.48 |
| 30 | 9.675 | 52.74 | 30.41 | 0.41 |
| 20 | 6.450 | 52.66 | 20.30 | 0.30 |
| 10 | 3.225 | 52.58 | 10.17 | 0.17 |
| 1 | 0.323 | 52.51 | 1.02 | 0.02 |

The correction necessary to be added to any reading ($s$) of the saccharimeter scale, as formulated from the above table, is equal very closely to $+ 0.02\,s - 0.0002\,s^2$. The percentage of glucose ($G$) corresponding to any scale reading ($s$) of the saccharimeter is, therefore, expressed by the formula

$$G = s + 0.02\,s - 0.0002\,s^2.$$

Some authorities have established the normal weights of sugars for 5, 10, 15, 20, and 25 per cent solutions. Landolt* gives as the normal weight of glucose for a 5 per cent solution 32.91 gms., for a 15 per cent solution 32.75 gms., and for a 25 per cent solution 32.50 gms., in which connection he states that, in weighing out the glucose-containing material for polarization, the chemist must select his normal weight according to the amount of glucose present. This, of course, involves a preliminary assay of the material under examination, which means practically doubling the work of analysis. A variable normal weight is, moreover, confusing, and a source of error. Wherever possible one fixed value should be given to the normal weight, the value to be selected (as in the case of sucrose) being that weight of chemically pure sugar, which dissolved to 100 true c.c. and polarized at 20° C. in a 200-mm. tube will give a constant reading of exactly 100 upon the saccharimeter. If in the use of such a normal weight with impure products, readings of less than 100 are obtained, the latter are corrected by a table or formula similar to that just given for glucose.

**Conversion of Saccharimeter Readings into Weight of Sugars.** — It is often desirable to express the equivalent of a saccharimeter reading, for a 200-mm. tube, in grams of a particular sugar in 100 c.c. This equivalent can be found by multiplying the value $a$ of the formulæ

* Landolt, "Das optische Drehungsvermögen" (1898), p. 448.

on page 194 by the angular rotation of 1 degree of the saccharimeter scale (page 145), thus:

1° angular rotation $D$ = 0.4785 gm. arabinose.
1° Ventzke sugar scale = 0.4785 × 0.34657 = 0.1658 gm. arabinose.
1° French sugar scale = 0.4785 × 0.21666 = 0.1037 gm. arabinose.
1° Wild sugar scale = 0.4785 × 0.13284 = 0.0635 gm. arabinose.

Owing to the lack of absolute agreement in the value of each saccharimeter scale in circular degrees, due to rotation dispersion, variation in quality of light, etc., the equivalent of 1 degree of a saccharimeter scale is best expressed as $\frac{1}{100}$ of the weight of sugar, which will give a reading of 100 degrees under the prescribed conditions of analysis (i.e. $\frac{1}{100}$ of its normal weight). The correction for concentration is afterwards applied as indicated above.

The approximate value of 1° V. for the more common sugars is given below.

*Weight of Sugar in* 100 *metric c.c.*

1° V. at 20° C. = 0.2600 gm. sucrose.
1° V. at 20° C. = 0.3225 gm. glucose.
1° V. at 20° C. = 0.1859 gm. fructose.
1° V. at 20° C. = 0.3286 gm. lactose hydrate.
1° V. at 20° C. = 0.1247 gm. maltose.
1° V. at 20° C. = 0.1655 gm. arabinose.
1° V. at 20° C. = 0.9100 gm. xylose.
1° V. at 20° C. = 0.2135 gm. galactose.
1° V. at 20° C. = 0.8645 gm. invert sugar.
1° V. at 20° C. = 0.1651 gm. raffinose hydrate.

**Use of One Normal Weight for All Sugars.** — For many laboratory purposes it is convenient to employ but one fixed normal weight for all saccharimetric work. In such cases the normal weight of sucrose is usually taken, the percentage of each particular sugar being calculated from the scale reading by means of an appropriate factor.

The constant polarizations in degrees Ventzke of a normal weight of 26.00 gms. of different sugars, when dissolved to 100 metric c.c. and polarized in a 200-mm. tube, are given in table XXXVI. The values are calculated only to the nearest 0.5 degree, which is sufficiently exact when the variations due to change in concentration are considered.

If no other optically active substances are present, the scale reading (V.°) of 26.00 gms. of the sugar-containing substance multiplied by 100 and divided by the corresponding polarizing power of the pure sugar will give the percentage of sugar present. Owing to the changes in specific rotation with varying concentration, the percentages thus calculated will not be absolutely exact.

TABLE XXXVI

*Giving Ventzke Reading of* 26.00 *gms. of Different Sugars in* 100 *c.c.*

| Sugar. | $[\alpha]_D^{20°}$ 26.00 gms. in 100 metric c.c. | Calculated reading V°. $\frac{[\alpha]_D^{20°} \times 100.}{66.5}$ |
|---|---|---|
| Sucrose | + 66.5 | +100 |
| Arabinose | +104.5 | +157 |
| Xylose | +19.6 | +29.5 |
| Glucose | +53.2 | +80 |
| Fructose | −93.2 | −140 |
| Invert sugar | −20.0 | −30 |
| Galactose | +81.8 | +123 |
| Maltose | +138.0 | +207.5 |
| Lactose ($H_2O$) | +52.5 | +79 |
| Raffinose (5 $H_2O$) | +104.5 | +157 |
| Raffinose (anhydride) | +123.2 | +185 |

## TECHNICAL METHODS OF SACCHARIMETRY

The saccharimeter is most generally employed in the analysis of products of the cane- and beet-sugar industry. It must be borne in mind, however, that the readings of the saccharimeter scale indicate percentages of sucrose only in cases where other constituents have no effect upon the scale reading; the results obtained with impure products are, therefore, more correctly expressed as degrees polarization or degrees sugar scale. For a more accurate determination of sucrose by the saccharimeter, the method of inversion must be used which will be described in the following chapter.

### *Methods for Polarizing Raw Sugars*

**Rules of the International Commission.** — The rules of the International Commission for Unifying Methods of Sugar Analysis* are as follows:

"In general all polarizations are to be made at 20° C.

"The verification of the saccharimeter must also be made at 20° C. For instruments using the Ventzke scale 26 grams of pure dry sucrose, weighed in air with brass weights, dissolved to 100 metric c.c. at 20° C. and polarized in a room, the temperature of which is also 20° C., must give a saccharimeter reading of exactly 100.00. The temperature of the sugar solution during polarization must be kept constant at 20° C.

"For countries where the mean temperature is higher than 20° C., saccharimeters may be adjusted at 30° C. or any other suitable tem-

* Proceedings of Paris Meeting, July 24, 1900.

perature, under the conditions specified above, provided that the sugar solution be made up to volume and polarized at this same temperature.

"In effecting the polarization of substances containing sugar employ only half-shade instruments.

"During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarizing Nicol is not warmed.

"As sources of light employ lamps which give a strong illumination such as triple gas burner with metallic cylinder, lens and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; sodium light.

"Before and after each set of observations the chemist must satisfy himself of the correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of the accuracy of his weights, polarization flasks, observation tubes and cover-glasses. (Scratched cover-glasses must not be used.) Make several readings and take the mean thereof, but no one reading may be neglected.

"In making a polarization use the whole normal weight for 100 c.c., or a multiple thereof, for any corresponding volume.

"As clarifying and decolorizing agents use either subacetate of lead, alumina cream, or concentrated solution of alum. Boneblack and decolorizing powders are to be excluded.

"After bringing the solution exactly to the mark at the proper temperature, and after wiping out the neck of the flask with filter paper, pour all of the well-shaken clarified sugar solution on a rapidly acting filter. Reject the first portions of the filtrate and use the rest, which must be perfectly clear for polarization."

**Methods of the New York Sugar Trade Laboratory.** — Details of manipulation for the above rules are left largely to individual preference or requirement. The course of operations pursued by the New York Sugar Trade Laboratory, where rapidity as well as accuracy is required, is as follows:

*Weighing.* — Twenty-six grams of sugar are weighed out in a nickel sugar dish provided with a counterpoise (Figs. 116 and 123). The sugar is stirred with a horn spoon and, approximately, the normal weight transferred to the dish. The final adjustment is then made with the dish upon the scale pan of the balance, a little sugar being added or removed until the exact weight is secured. The danger of spilling sugar upon the scale pan during the weighing is thus largely avoided. The weighing is performed as rapidly as possible to avoid loss from

evaporation of moisture and does not usually consume more than a minute of time.

*Transferring.* — The 26 gms. of sugar in the nickel dish are poured into a large funnel placed in a sugar flask; any sugar adhering to the dish and funnel is then washed into the flask with distilled water, the funnel being thoroughly rinsed inside and outside around the bottom to insure the complete removal of all sugar to the flask. From 50 to 60 c.c. of water are sufficient to effect the transference.

The funnels employed in transferring the sugar are of German silver, and have a mouth 4 in. ($11\frac{1}{2}$ cm.) in width and 3 in. (9 cm.) in depth, and a stem 3 in. (9 cm.) in length. The inner diameter of the

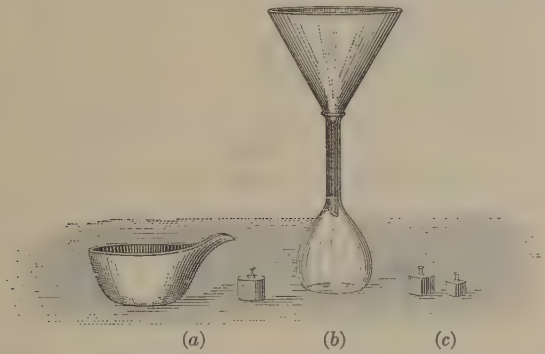



Fig. 123. — (*a*) Nickel weighing dish and counterpoise. (*b*) Funnel for transferring sugar. (*c*) Normal and half-normal metric c.c. sugar weights.

stem ($8\frac{1}{2}$ mm.) is sufficiently large to allow a free passage of the sugar into the flask and the outer diameter (10 mm.) sufficiently small to allow the escape of air from the flask (see Fig. 123).

*Dissolving.* — The solution of the sugar in the flasks is performed by means of a mechanical shaker. The machine employed in the New York Sugar Trade Laboratory is a modification by the author of the Camp shaker used in iron and steel laboratories. (Fig. 124.)

The metal disk of this shaker is replaced by a circular piece of oak $\frac{7}{8}$ in. thick, of the same diameter and of about the same weight, and containing 12 holes $2\frac{1}{8}$ in. in diameter, each large enough to accommodate the bottom of a sugar flask. Six extra gripping devices are inserted in the collar of the shaker, thus giving 12 grips in all to hold the necks of the flasks. The collar is adjusted so as to bring the grips at the right height and exactly over the centers of the circular holes in the wooden disk. The bottom of the flasks are inserted in the holes, and,

by pressing the necks against the springs of the grips, the flasks are snapped quickly and securely into position. The shaker is connected with a small $\frac{1}{8}$ horse-power electric motor, provided with a rheostat, and the speed of its driving wheel gradually brought up to 120 to 130 revolutions per minute. At this speed, solution of sugar in the flasks, using 50 to 60 c.c. of water, is effected in from 5 to 10 minutes, according to the size of grain, stickiness of sample, etc. If too much water is used in transferring the sugar, less motion is given to the body of the liquid, and a longer time is required to effect solution.

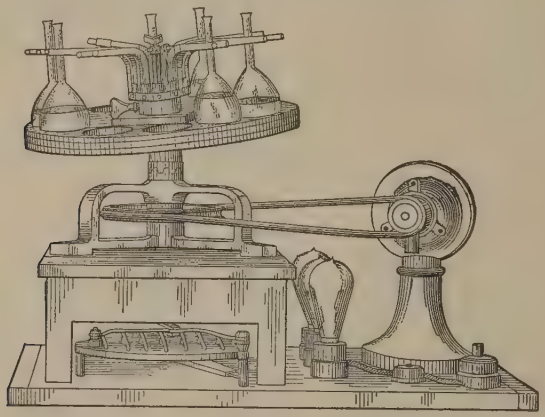


Fig. 124. — Mechanical shaker for dissolving sugars.

*Clarifying.* — The solution is then clarified with the requisite amount of lead subacetate solution (sp. gr. 1.25), but no more than the amount necessary to secure a clear polariscope reading is ever employed. As a rule not over 1 c.c. of the lead subacetate solution is used for Java, Peruvian, and high-grade centrifugal sugars, not over 1 to 2 c.c. for muscovado sugars, from 2 to 6 c.c. for molasses sugars, and 3, 4, and 5 c.c. for Philippine mat sugars according to grade. Excess of lead solution increases the polarization very markedly and strict observance is paid to the rule of minimum quantity necessary for clarification. After the lead solution 2 c.c. of alumina cream are added, the contents of the flask are well mixed and the volume of liquid made up to 100 c.c., after allowing sufficient time for any air bubbles to arise which may have been occluded in the lead precipitate. Foam and air bubbles, adhering to the surface of the liquid in the neck of the flask, are broken up with a fine spray of ether before adjusting the

volume to the graduation mark. A small bulb atomizer (Fig. 125) is convenient for removing foam.

The distilled water used in all the work is supplied through rubber tubing from a large bottle placed at an elevation above the laboratory table. The outlets of the rubber tubes are fitted with pinch cocks and glass tips of large and fine opening, the former being used for transferring the sugar and the latter for setting the meniscus. The adjustment of the meniscus to the graduation mark is the same as that used in calibration (Fig. 119). The distilled water used for solution is kept as nearly as possible at 20° C., and the completion of the volume of sugar solution to 100 c.c. is always made with the contents of the flask at this temperature.

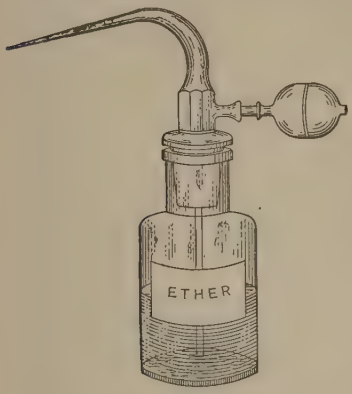


Fig. 125. — Ether atomizer.

*Filtering.* — The contents of the flasks after thorough mixing are poured upon plaited filters in stemless funnels resting in $\frac{1}{2}$-pint jars or cylinders (Fig. 120). All glassware is thoroughly cleaned and dried before using. The plaited filters, which are large enough to hold the entire contents of the flask, are kept in a large desiccator until ready for use. The funnels are covered with watch glasses during the filtration to prevent concentration of liquid through evaporation. The first runnings (10 to 15 c.c.) of the filtrate are rejected and the remainder used for polarization.

### *Methods for Polarizing Juices, Sirups, Molasses, Massecuites, etc.*

The method of polarization just described for raw sugars may be applied with minor modifications to the examination of sugar-cane, sugar-beet, sorghum, and other plant juices, sirups, molasses, massecuites, and all other products which are mostly soluble in water.

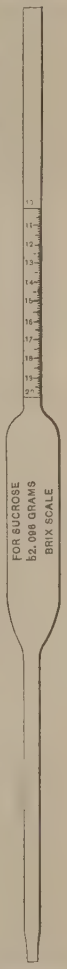


Fig. 126.— Spencer's sucrose pipette.

**Sucrose Pipette.** — In the analysis of sugar-containing juices the work of analysis may be lightened considerably by the use of Spencer's

or Crampton's sucrose pipette shown in Fig. 126. This pipette is graduated upon the stem with divisions, divided into tenths, reading from 5 to 25. The pipette is so calibrated that the volume of juice delivered from the division upon the stem, which corresponds to its degrees Brix, is exactly a double normal weight. The pipette is constructed either for Mohr cubic-centimeter or true cubic-centimeter flasks, delivering 52.096 gms. and 52.000 gms. of juice respectively. The method of employing the pipette is thus described by Spencer.*

"Determine the density of the juice with a Brix hydrometer, noting the degree Brix without temperature correction. Fill the pipette with juice to the mark corresponding with its observed degree Brix, and discharge it into a 100-c.c. flask. Add 3 to 5 c.c. of diluted lead-subacetate solution, complete the volume to 100 c.c. with water, mix thoroughly and filter the contents of the flask. Polarize the filtrate, using a 200-mm. tube, and divide the polariscope reading by 2 to obtain the percentage of sucrose. The juice should not be expelled from the pipette by blowing, and sufficient time should be allowed for thorough drainage. Each pipette should be tested when received from the maker, and in regular work should be used under the conditions of the test. The pipette may be conveniently checked against a balance by delivering a measured quantity of juice into a tared capsule and weighing it. The uncorrected degree Brix and juice of the temperature of the Brix observation must be used. If the hydrometer and pipette are correct at the parts used, the juice delivered should weigh 52.096 gms. (or 52.00 gms. for true cubic centimeters).

"It is not advisable to use these pipettes with liquids of a higher density than 25 degrees Brix or of greater viscosity than cane juice. These pipettes are usually used in the analysis of miscellaneous samples of juice and in the rapid testing of diluted massecuites and molasses for guidance in the vacuum-pan work. They should be frequently cleaned with a strong solution of chromic acid in sulphuric acid."

For the analysis of highly concentrated sugar products, such as sirups, molasses, massecuites, etc., the normal weight of substance is weighed out as with raw sugar. In case of very dark-colored molasses and massecuites, it is often necessary to make the normal weight of substance after clarification up to 200 c.c. instead of 100 c.c. in order to reduce the depth of color sufficiently to polarize in a 200-mm. or, even at times, in a 100-mm. tube. The reading thus obtained is multiplied by 2 (or if polarization is made in a 100-mm. tube by 4) to obtain the true direct polarization.

* Spencer's "Handbook for Cane Sugar Manufacturers" (4th Ed.), p. 122.

### Clarifying Agents and Errors Attending Their Use

In the clarification of dark-colored molasses and other sugar-house products a much larger amount of clarifying agent must be used than is necessary with raw sugars, juices, and other substances of high purity. The employment of excessive quantities of clarifying agent introduces, however, serious errors in the work of polarization. These errors for convenience will be considered under the following heads:

I. Errors due to the volume of precipitated impurities.
II. Errors due to precipitation of sugars from solution.
III. Errors due to change in specific rotation of sugars.

The influence of these errors will first be considered in connection with the different acetates of lead which are the salts most generally used for clarification.

**Acetates of Lead.** — Four well characterized acetates of lead have been isolated in the crystalline form. These are (1) the normal or neutral acetate of lead $Pb(CH_3COO)_2$; (2) the ordinary basic or subacetate of lead $3\ Pb(CH_3COO)_2,\ 2\ PbO$; (3) the monobasic acetate of lead $Pb(CH_3COO)_2,PbO$; (4) the dibasic acid of lead $Pb(CH_3COO)_2,\ 2\ PbO$. The clarifying power of solutions of these acetates is in general proportionate to the content of basic PbO. The normal acetate, although deficient in decolorizing power and unsuited for the clarification of dark-colored products for polariscopic readings, has certain advantages in that it does not precipitate reducing sugars from solution and does not form soluble lead-sugar compounds of different specific rotation. For these reasons the neutral acetate of lead should be employed for clarifying wherever possible in preference to the basic salt.

**Neutral Lead-acetate Solution.** — In preparing the neutral acetate of lead reagent, a concentrated solution of commercial lead acetate (sugar of lead) is made, any free alkali or acid neutralized with acetic acid or sodium hydroxide, and the liquid diluted to a density of 30 degrees Bé. (54.3 degrees Brix or 1.2536 sp. gr. $\frac{20°}{4°}$). The solution is filtered and kept in a stock bottle ready for use.

**Lead-subacetate Solution.** — Upon digesting litharge with normal acetate of lead solution varying amounts of lead oxide are dissolved according to the time and temperature of digestion. Numerous methods are employed for preparing lead-subacetate reagent. The following examples are given:

I. *Concentrated Solution.**—Heat, nearly to boiling, for about half an hour, 860 gms. of neutral lead acetate, 260 gms. of litharge, and

* Spencer's "Handbook for Cane Sugar Manufacturers," p. 229.

500 c.c. of water. Add water to compensate for the loss by evaporation. Cool, settle, and decant the clear solution. The solution may be prepared without heat, provided the mixture is set aside several hours with frequent shaking.

*Dilute Solution.* — Proceed as described above, using, however, 1000 c.c. of water. The solution should be diluted with cold, recently boiled distilled water to 54.3 degrees Brix (30 degrees Bé., or 1.2536 sp. gr. $\frac{20°}{4°}$).

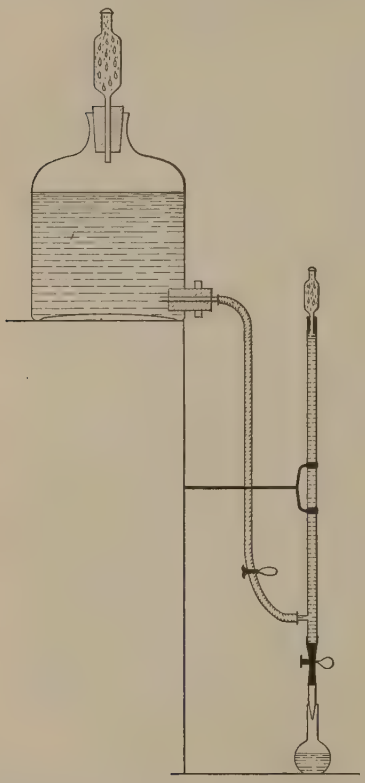


Fig. 127. — Stock bottle and burette for lead subacetate solution.

II.* Boil 430 gms. of normal lead acetate, 130 gms. of litharge, and 1000 c.c. of water for half an hour. Allow the mixture to cool and settle and dilute the supernatant liquid to 1.25 sp. gr. with recently boiled distilled water.

III.† Treat 600 gms. of neutral lead acetate and 200 gms. of litharge with 2000 c.c. of water. After standing 12 hours in a warm place with occasional shaking, the solution is filtered and the filtrate stored in tightly stoppered bottles. The solution thus prepared must show a strongly alkaline reaction and have a specific gravity of 1.20 to 1.25 (at 17.5° C.) with a content of about 20 per cent PbO.

IV. Lead-subacetate solution may also be prepared by dissolving the solid basic salt (see page 214). The concentrated solution is diluted with distilled water to a specific gravity of 1.25.

Stock solutions of lead subacetate, both in bottle and burette, should be protected by a soda-lime tube from the carbon dioxide of the air to prevent deposition of lead carbonate (see Fig. 127).

* "Methods of Analysis A. O. A. C.," Bull. 107 (revised), U. S. Bur. of Chem., p. 40.

† Frühling's "Anleitung," p. 457.

*I. Errors of Clarification Due to Volume of Precipitated Impurities*

Since all sugar solutions after clarification with lead subacetate, or other means, are made up to a definite volume, the space occupied by the precipitated impurities will cause the sugar solution to occupy a somewhat smaller volume than that of the flask in which the solution was made up. An increase in concentration and also in polarization is the result.

**Scheibler's Method of Double Dilution.** — Several methods have been devised for estimating the extent of this error. The first to be described is Scheibler's* method of double dilution. In this method a normal weight of product is dissolved in water, clarified with a measured volume of lead subacetate, the volume completed, and solution filtered and read in the usual way. A second normal weight of product is then weighed out, clarified with the same volume of reagent as before and the solution made up to twice the volume of the previous experiment. The second solution is filtered and polarized as before. The true polarization ($P$) is then calculated as follows:

Let $P_1$ be the polarization of the first solution made up to volume $V$, and $P_2$ the polarization of the second solution made up to volume $2\,V$. Let $v$ be the volume of the precipitated impurities which is assumed to be the same in both experiments. The normal weight in the second solution may be considered to be divided as follows: one half dissolved in volume $V$ free from precipitate, the reading of which would be $\frac{P}{2}$, and one half dissolved in volume $V$ containing precipitate, the reading of which would be $\frac{P_1}{2}$. The sum of these quantities divided by 2 is the value of $P_2$, or

$$\frac{\frac{P}{2} + \frac{P_1}{2}}{2} = P_2$$

whence $P = 4\,P_2 - P_1$. In other words the true polarization is equal to four times the polarization of the diluted solution less the polarization of the undiluted solution.†

* Z. Ver. Deut. Zuckerind., **25**, 1054.

† The true polarization is also expressed in other ways as: multiply reading of dilute solution by 2, subtract the product from reading of undiluted solution; twice the remainder subtracted from reading of undiluted solution will give the true polarization: or the difference between the reading of the undiluted solution, and twice the reading of diluted solution subtracted from twice the reading of the diluted solution will give the true polarization.

*Example.* — Polarization of 26 gms. raw sugar, dissolved in water, clarified with 2 c.c. lead subacetate and made to 100 c.c. = 94.2 ($P_1$).

Polarization of 26 gms. same sugar, dissolved in water, clarified with 2 c.c. lead subacetate and made to 200 c.c. = 47.0 ($P_2$).

True polarization ($P$) = $(47.0 \times 4) - 94.2 = 93.8$.

The volume $v$ occupied by the precipitated impurities is calculated as follows. The reading $P_1$ of the undiluted solution is equal to $\frac{V \times P}{V - v}$, whence $v = \frac{V(P_1 - P)}{P_1}$.

*Example.* — Required the volume of the lead precipitate in the previous example.

Substituting the values for $V$, $P$ and $P_1$, we obtain

$$v = 100 \frac{(94.2 - 93.8)}{94.2} = 0.42 \text{ c.c.}$$

The method of Scheibler owing to its rapidity and ease of execution has been very widely used for correcting polarizations for the error due to volume of the lead precipitate. The method is open to several objections. It is not probable that the volume of the precipitate is exactly the same in the dilute as in the undiluted solution, but the principal objection against the method is the very large multiplication of any error made in reading the diluted solution.

**Sachs's Method of Correcting Precipitate Error.** — The method devised by Sachs* in 1880 for determining the error due to volume of precipitate was intended to obviate the errors of Scheibler's method. In the Sachs method the precipitate of impurities obtained in the clarification of the sugar solution is washed with cold and hot water until all sugar is removed. The precipitate is then transferred to a 100-c.c. flask, a one-half normal weight of sucrose added, the latter dissolved and the volume completed to 100 c.c. The solution is mixed, filtered, and polarized in a 400-mm. tube. The volume of precipitate is then calculated as follows: Let $P$ = the true polarization of the sucrose used and $P_1$ = the polarization of the sucrose with precipitate. The volume ($v$) of precipitate is then found by the equation

$$v = \frac{100(P_1 - P)}{P_1}.$$

*Example.* — A normal weight of granulated sugar dissolved to 100 c.c. polarized 99.8 in a 200-mm. tube.

A one-half normal weight of the same sugar + lead precipitate dissolved to 100 c.c. polarized 100.25 in a 400-mm. tube. Volume of precipitate ($v$) = $100 \frac{(100.25 - 99.8)}{100.25} = 0.45$ c.c.

* Z. Ver. Deut. Zuckerind., **30,** 229.

Knowing the volume ($v$) of lead precipitate, the true polarization ($P$) of a product may be determined by the equation $P = \frac{VP_1 - vP_1}{V}$, or when $V = 100$, $P = \frac{100\,P_1 - vP_1}{100}$.

*Example.* — The polarization of a raw sugar (26 gms. to 100 c.c.) was 96.20 ($P_1$). The volume of the lead precipitate by Sachs's method was 0.22 c.c. ($v$). The true polarization ($P$) of the sugar $= \frac{100 \times 96.2 - 0.22 \times 96.2}{100} = 95.99$.

The method of Sachs has been modified as follows. Instead of making a polarization with the washed precipitate the latter is first dried. From the weight and specific gravity of the dried lead precipitate the volume is calculated $\left(v = \frac{w}{\text{sp. gr.}}\right)$ and from the volume the true polarization is determined by means of the preceding formula.

The specific gravity of the dried lead precipitates of raw cane sugars was determined by Wiechmann* by weighing in a pycnometer with benzine. The results of Wiechmann are given in Table XXXVII.

TABLE XXXVII

*Giving Specific Gravity and Volume of Lead Precipitates from 26 gms. of Different Raw Cane Sugars*

| Sugar. | Weight of precipitate in grams. | Specific gravity $H_2O = 1.00$. | Volume in cu. centimeters |
|---|---|---|---|
| Jamaica Muscovado | 0.4559 | 1.88 | 0.24 |
| Maceio Muscovado | 0.8112 | 1.65 | 0.49 |
| San Domingo centrifugal | 0.2525 | 2.91 | 0.09 |
| Sandwich Island centrifugal | 0.1378 | 2.84 | 0.05 |
| San Domingo concrete | 1.0139 | 3.80 | 0.27 |
| Porto Rico molasses sugar | 0.8959 | 4.35 | 0.21 |
| Sandwich Islands | 1.0195 | 4.38 | 0.23 |
| Cebu mats | 1.5400 | 2.17 | 0.71 |
| Manila mats | 1.3350 | 2.22 | 0.60 |

Similar results by Horne are given in Table XXXVIII. The method employed by Horne† consists in weighing the freshly washed precipitate in a calibrated pycnometer filled to the mark with distilled water; the precipitate is then washed upon a weighed filter, dried and weighed.

The methods, which are based upon the separation and examination of the washed lead precipitate, throw much light upon the errors

* Proc. Fifth Int. Cong. Applied Chem. (Berlin, 1904) III, 118.

† J. Am. Chem. Soc., **26,** 186.

of clarification; they are not adapted, however, to practical work owing to the large amount of time and labor involved.

**Horne's Method of Dry Defecation.** — A third method of eliminating the volume of precipitate error is Horne's* process of dry defecation. The method is thus described by its author:

"The normal weight of sugar is dissolved in water in a 100-c.c. flask and made up to the mark without defecation. The concentration is thus at exactly the proper degree. It now remains to defecate the solution properly by precipitating the impurities in such a way as to produce the minimum change in the concentration of the solution of sucrose. This is accomplished by adding to the 100 c.c. of liquid small quantities of powdered anhydrous lead subacetate until the impurities are nearly all precipitated. This point is as easily determined as in the defecation by a solution of the same salt. The organic and mineral-acid radicals in the solution combine with and precipitate the lead and lead oxide of the dry salt, while the acetic-acid radical of the lead subacetate passes into solution to combine with the bases originally united to the other acid radicals."

Results obtained by Horne upon 12 raw cane sugars are given in Table XXXVIII, and show a very close agreement between the corrected polarization by Sachs's method and the polarization by dry defecation.

TABLE XXXVIII

| | Grade, country. | Ordinary polarization. | Specific gravity of precipitate. | Volume of precipitate. | Corrected polarization. | Dry lead polarization. |
|---|---|---|---|---|---|---|
| | | | | c.c. | | |
| 1 | Centrifugal | 95.0 | 2.98 | 0.10 | 94.9 | 94.9 |
| 2 | Centrifugal (mixed samples) | 94.5 | ..... | 0.0765 | 94.43 | 94.4 |
| 3 | Centrifugal, Trinidad | 96.95 | 2.91 | 0.0378 | 96.91 | 96.95 |
| 4 | Centrifugal, Java | 97.425 | 2.30 | 0.0884 | 97.33 | 97.375 |
| 5 | Muscovado, St. Croix | 85.8 | 1.91 | 0.4118 | 85.45 | 85.5 |
| 6 | Molasses, Cuba | 89.4 | 3.20 | 0.39 | 89.05 | 89.0 |
| 7 | Molasses | 89.225 | 2.85 | 0.4204 | 88.85 | 88.85 |
| 8 | Molasses | 86.45 | 1.96 | 0.7108 | 85.84 | 85.95 |
| 9 | Molasses | 90.675 | 3.20 | 0.3204 | 90.39 | 90.45 |
| 10 | Molasses | 89.35 | ..... | 0.8500 | 88.59 | 88.775 |
| 11 | Molasses | 89.4 | 3.01 | 0.4554 | 88.99 | 89.0 |
| 12 | Molasses, Cuba | 88.4 | 2.64 | 0.4924 | 87.97 | 88.0 |

Horne's method has been tested by a number of chemists upon raw cane sugars with results very similar to the above. Pellet,† how-

* J. Am. Chem. Soc., **26**, 186.

† Bull. assoc. chim. sucr. dist., **23**, 285.

ever, has criticized the method principally upon the ground that the increase in polarization due to the volume of precipitate is not as great as calculated, owing to the decrease in polarization caused by the retention of sucrose in the precipitate, this retention error frequently more than counterbalancing the error due to volume of precipitate. Subsequent results by Horne* and other chemists show, however, that there is no appreciable retention of sucrose when the dry lead reagent is used in minimum amounts. Another objection by Pellet, that only part of the lead salt acts and that the rest passes into solution, thus increasing the volume and diminishing the polarization, deserves consideration.

With the higher grade of sugar-house products there is no difficulty in securing a satisfactory clarification with a minimum amount of the dry lead salt, the lead dissolved being immediately precipitated and but very little remaining in solution. With low-grade sugars, molasses, etc., the case is otherwise. If dry lead subacetate, or subacetate solution, be added to a solution of such products to the point of satisfactory clarification a considerable amount of lead salt will usually remain dissolved. The rule of adding the powdered salt until no more precipitate forms is not always a criterion of the absence of lead in the filtrate. When subacetate is added to solutions of low purity the first portions of lead are completely precipitated; then comes a point where with the formation of additional precipitate a small amount of lead remains in solution; the amount of the latter continues to increase until at the point where no more precipitate is formed nearly all of the lead added remains dissolved. (See Table XXXIX.) With very low grade products there is therefore a danger of the dry lead salt increasing the volume of solution; whether this increase will cause a lowering of the polarization or not will depend upon the character of the product. With low-grade sugar-cane products the error due to increase in volume of solution may be more than counterbalanced by the precipitation of levorotatory fructose.

In the following experiments by Hall† in the New York Sugar Trade Laboratory the effect of increasing amounts of dry lead subacetate upon the polarization of a Philippine mat sugar was studied. The quantity of lead in the clarified filtrates was determined and the dilution calculated by allowing an increase of 0.22 c.c. in volume for 1 gm. of dry subacetate dissolved in 100 c.c. of solution.

* J. Am. Chem. Soc., **29**, 926.

† Bull. 122, U. S. Bur. of Chem., p. 225.

TABLE XXXIX

*Showing Estimated Dilution of a Sugar Solution by Dry Lead Subacetate*

| Clarifying agent. | Amount of clarifying agent used. | In 100 c.c. filtrate. | | Estimated dilution. | Polarization. |
|---|---|---|---|---|---|
| | | PbO. | Pb subacetate. | | |
| | | grams. | grams. | c.c. | |
| Subacetate solution.. | 3.0 c.c. | 0.2678 | ..... | ..... | 86.70 |
| Dry subacetate..... | 0.5 gm. | Trace | ..... | Trace | Too dark to read. |
| Dry subacetate..... | 1.0 gm. | 0.1530 | (0.20) | 0.05 | 86.50 |
| Dry subacetate..... | 2.0 gms. | 0.7203 | (0.94) | 0.20 | 86.60 |
| Dry subacetate..... | 4.0 gms. | 2.1078 | (2.73) | 0.60 | 86.50 |

It is noted that with an estimated dilution of 0.2 c.c. instead of a decrease in polarization, as would be expected, there is an increase. With an estimated dilution of 0.6 c.c. the reading is the same as that first obtained, so that the combined effect of the dry lead upon the precipitation of fructose and upon the lowering of the rotation of the fructose in solution is seen to be most pronounced. With sugar-cane products the use of dry lead subacetate to the point of satisfactory clarification would seem to involve no decrease in polarization. With low-grade sugar-beet and other products, which are comparatively free from fructose, there is however a danger of too low polarization since there is no compensating influence for the dilution caused by the excess of lead subacetate dissolved.

In using dry lead subacetate for defecation the chemist must be certain of the composition of his preparation. The powdered salt must be dry and should contain the requisite amount of basic lead. Some samples of dry lead subacetate sold by the trade have been found by the author to consist almost entirely of the normal acetate. A very pure anhydrous lead subacetate is manufactured at present having closely the formula, 3 $Pb(C_2H_3O_2)_2$, 2 PbO. A sample of such a preparation analyzed at the New York Sugar Trade Laboratory gave the following results:

| | Total Pb. | Basic Pb. |
|---|---|---|
| | Per cent. | Per cent. |
| Found..... | 73.00 | 30.03 |
| Theory for 3 $Pb(C_2H_3O_2)_2$, 2 PbO.... | 72.84 | 29.14 |

The slightly higher results found for total and basic lead are probably

due to the presence of a small amount of the monobasic acetate, $Pb(C_2H_3O_2)_2$, PbO.

A solution of lead subacetate of 1.259 sp. gr., as employed for clarification in the wet way, was found to contain 0.2426 gm. total Pb per 1c.c. One-third gram dry salt is therefore equivalent to 1 c.c. subacetate solution in clarifying power. A low-grade sugar requiring 6 c.c. of subacetate solution of the above strength for clarification would accordingly need 2 gms. of salt for dry defecation.

The dry subacetate of lead employed in sugar analysis should be finely ground in order that it may be acted upon quickly and completely by the dissolved impurities. The tendency to form insoluble crusts upon the powdered grains of dry salt has been noted by Horne, especially in refinery products subjected to the influence of bone black. In such cases Horne recommends the addition of a little dry sand with the powdered lead salt; the particles of sand in shaking will grind off the crusts of insoluble matter and allow the lead to be completely acted upon.

*II. Errors of Clarification due to Precipitation of Sugars from Solution*

In the absence of free alkalies sucrose is not precipitated from solution by lead subacetate. Reducing sugars, however, are precipitated by solutions of basic lead salts. This precipitation does not occur with the amounts of lead used in ordinary clarification except in presence of those salts or acids which form insoluble lead compounds* (as chlorides, sulphates, phosphates, carbonates, oxalates, tartrates, malates, etc.). Whether this precipitation of reducing sugars is due to simple occlusion or to the formation of insoluble sugar-lead complexes is not definitely known.

The extent to which the common reducing sugars, glucose and fructose, are precipitated by different lead clarifying agents, has been investigated by Bryan.† Separate solutions of glucose and fructose were prepared, using 5 gms. of sugar with 1 gm. each of magnesium sulphate and ammonium tartrate. To 50 c.c. of this solution the clarifying agent was added and the volume made up to 100 c.c. After filtering, the excess of lead was removed with potassium oxalate, and the sugar in solution determined by Allihn's method. The results of Bryan's experiments are given in the following table.

* Prinsen Geerligs, Deut. Zuckerind., **23**, 1753.

† Bull. 116, U. S. Bur. of Chem., p. 73.

TABLE XL

*Showing Precipitation of Glucose and Fructose by Basic Lead Salts*

| Clarifying agent. | Amount per 100 c.c. of solution. | Glucose precipitated. | Fructose precipitated. |
|---|---|---|---|
| | | Per cent of total. | Per cent of total. |
| Neutral lead acetate solution | 3.5 c.c. | 0.93 | 0.00 |
| Neutral lead acetate solution | 7.0 c.c. | 0.84 | 0.00 |
| Lead subacetate solution | 3.5 c.c. | 3.35 | 8.03 |
| Lead subacetate solution | 7.0 c.c. | 8.34 | 19.91 |
| Dry lead subacetate | 1.0 gm. | 3.85 | 14.93 |
| Dry lead subacetate | 2.5 gms. | 17.48 | 35.33 |
| Basic lead nitrate solution | 4.0 c.c. | 6.27 | 13.84 |
| Basic lead nitrate solution | 8.0 c.c. | 5.61 | 25.12 |

It is seen that neutral lead acetate precipitates but very little reducing sugar, whereas the basic lead salts remove a large percentage of both glucose and fructose, the latter sugar, however, in more than double the amount. This precipitation of reducing sugars during clarification has a most marked effect upon the polarization, the removal of glucose from solution diminishing the dextrorotation, and that of fructose the levorotation. The greater precipitation of fructose in mixtures with sucrose and glucose, as in the clarification of sugar-cane products, jellies, jams, etc., causes an increase in the dextrorotation, frequently exceeding 1° Ventzke. The precipitation of reducing sugars, while of no consequence as regards the saccharimetric or gravimetric determination of sucrose, is of the greatest importance when the valuation of a product is based upon the polarization alone, or upon a determination of reducing sugars.

*III. Errors of Clarification due to Change in Specific Rotation of Sugars*

**Action of Lead Subacetate on Rotation of Sucrose.** — The results of Müntz,* Weisberg,† Svoboda,‡ Gröger§ and other investigators show no perceptible influence of basic lead acetate upon the specific rotation of sucrose in aqueous solution. Recent experiments by Bates and Blake‖ indicate, however, a very perceptible influence in case the lead reagent is used in large excess. The following table, showing the loss and gain in polarization for a normal weight of pure sucrose, is taken from the work of Bates and Blake.

* J. fabr. sucre., **17,** 25.
† Sucrerie Belge, **16,** 407.
‡ Z. Ver. Deut. Zuckerind., **46,** 107.
§ Oest. Ung. Z. Zuckerind., **30,** 429.
‖ Bull. U. S. Bur. of Standards, **3** (1), p. 105.

TABLE XLI

| Number of cubic centimeters of basic lead solution (1.25 sp. gr.) added. | Difference in degrees Ventzke between similar solutions, one with the other without, basic lead acetate. |
|---|---|
| 0.5 | −0.09 |
| 1.0 | −0.13 |
| 2.0 | −0.13 |
| 3.0 | −0.08 |
| 4.0 | −0.06 |
| 5.0 | −0.03 |
| 6.0 | 0.00 |
| 7.0 | +0.05 |
| 8.0 | +0.09 |
| 10.0 | +0.19 |
| 15.0 | +0.29 |
| 20.0 | +0.45 |
| 25.0 | +0.58 |
| 30.0 | +0.62 |
| 35.0 | +0.77 |
| 40.0 | +0.77 |
| 63.0 | +0.95 |

The + sign indicates that the solution containing the lead subacetate gives the higher polarization, and conversely for the − sign.

**Action of Lead Subacetate on Rotation of Fructose.** — While the specific rotation of sucrose under the ordinary conditions of analysis is not modified sufficiently by subacetate of lead to introduce serious errors, the case is otherwise with fructose. Gill* first showed, in 1871, that the specific rotation of fructose was greatly diminished by the presence of lead subacetate, this decrease being so great that in presence of sufficient basic lead the rotation of invert sugar ($[\alpha]_D^{20} = -20$) was changed to the right. This change in rotation is due to the formation of soluble dextrorotatory lead fructosate, the presence of which, even in small amounts, is sufficient to reduce the figure for the rotation of fructose ($[\alpha]_D^{20} = -92$) below that of glucose ($[\alpha]_D = +52.5$). Gill† showed that the error due to formation of soluble lead fructosate could be entirely avoided by adding acetic acid to the point of acidity, thus decomposing the soluble lead fructosate into lead acetate and free fructose of normal specific rotation. In case the soluble lead fructosate is not decomposed by some precipitating agent of lead, acetic acid

* Z. Ver. Deut. Zuckerind., **21** (1871), 257.

† Loc. cit. See also "Spencer's Handbook for Cane Sugar Manufacturers" (4th Ed.), p. 88; Edson, Z. Ver. Deut. Zuckerind., **40**, 1037; Pellet, Bull. assoc. chim. sucr. dist., **14**, 28, 141.

should be added to weak acidity before making up the volume of the clarified solution to 100 c.c. for the direct polarization of low-grade fructose containing products.

*Miscellaneous Methods of Clarification*

Numerous modifications of the lead process of clarification have been proposed as a means of reducing or eliminating the several sources of error just mentioned. Freshly precipitated lead carbonate, lead chloride, and lead nitrate have been employed as clarifying agents, but with only indifferent success. Two methods of lead clarification, which have found considerable favor in France and Austria, should, however, be mentioned in addition to the processes previously described. These are Zamaron's method by means of hypochlorite of lime and neutral lead acetate, and Herles's method by means of basic lead nitrate.

**Zamaron's* Method of Clarification with Hypochlorite.** — 625 grams of dry commercial bleaching powder are thoroughly ground up in a large mortar with 1000 c.c. of water. The mass is squeezed out in a sack and the extract filtered through paper. The solution thus obtained (700 c.c. to 800 c.c. of about 18° Bé.), is preserved in a stoppered bottle of dark glass away from the light.

The solution to be clarified is treated with a few cubic centimeters of the hypochlorite solution, sufficient to effect decolorization, and then a few cubic centimeters of neutral lead acetate solution are added. There is usually a slight rise in temperature after addition of the clarifying agents so that the solution must be recooled before making to volume.

The Zamaron process secures usually a good clarification, does not precipitate reducing sugars, and forms no objectionable lead sugar compounds. The chief fault of the method is the volume of precipitate error, which in this case is augmented by the formation of considerable lead chloride.

**Herles's† Method of Clarification with Basic Lead Nitrate.** — Dissolve 100 grams of solid sodium hydroxide in 2000 c.c. of water; a second solution is prepared by dissolving 1000 gms. of neutral lead nitrate in 2000 c.c. of water. Upon mixing equal volumes of the two solutions basic lead nitrate is precipitated according to the equation

$$\underset{\text{Lead nitrate}}{2\,Pb(NO_3)_2} + 2\,NaOH = \underset{\text{Basic lead nitrate}}{Pb(NO_3)_2.Pb(OH)_2} + 2\,NaNO_3$$

* Fribourg's "Analyse chimique," p. 129.

† Z. Zuckerind., Böhmen, **13,** 559; **14,** 343; **21,** 189.

The precipitated basic lead nitrate is washed free from sodium compounds and then mixed with water to a cream, in which form it may be used for clarification.

The clarification is performed more commonly by forming the basic nitrate within the solution to be clarified. This is done by first adding a measured quantity of the lead-nitrate solution (1 c.c. to 15 c.c. according to depth of color) and then, after mixing, an equal volume of the sodium hydroxide solution. After shaking, the solution is made to volume, well mixed, and filtered. Care must be taken that the reaction of the solution is not alkaline after mixing; this is best provided for by testing the two solutions against one another before using.

Formation of the basic lead nitrate within the solution gives usually a much better clarification than addition of the washed cream, but has the disadvantage of introducing considerable sodium nitrate, which, if present in large quantity, will affect the rotation of the sugars.

The basic lead nitrate method gives an exceedingly brilliant clarification. The process is open, however, to the same errors as basic lead acetate. There is first the volume of precipitate error, which is further augmented by the copious bulk of the basic lead nitrate itself; and secondly there is a precipitation of reducing sugars as shown by the results of Bryan in Table XL.

The numerous errors incident to the use of basic lead compounds in clarification have led chemists to seek other means of decolorizing solutions for polarization. It is impossible, as well as unnecessary, to take up all the processes which have been devised to accomplish this end. Two of these methods, however, should be described: (1) Decolorization by means of bone black or blood charcoal; (2) Decolorization by means of hydrosulphites, sulphoxylates, etc.

**Decolorization of Sugar Solutions by means of Bone Black.** — The use of bone black as a decolorizing agent in sugar refineries is well known. The same substance in a more finely divided specially prepared form is employed at times as a decolorizer in sugar analysis.

*Purification of Bone Black.* — If purified animal charcoal (preferably blood charcoal) has not been obtained from the dealer the chemist may purify the commercial product as follows: The char is finely ground in a mortar and then digested several hours in the cold with dilute hydrochloric acid. The acid is then decanted, the char brought upon a filter and washed with distilled water until all traces of hydrochloric acid are removed. After drying in a hot-air oven, the char is heated to dull redness in a covered porcelain crucible, and then, after cooling sufficiently, placed while still warm in a dry stoppered bottle.

Several methods are followed in the employment of animal charcoal for decolorizing. One very common practice is to make up the solution to volume and shake thoroughly with a small quantity of charcoal, using from 0.5 to 3 gms. according to depth of color. The contents of the flask are then poured upon a dry filter and the filtrate taken for polarization.

*Absorption Error of Bone Black.*—In the above method of decolorizing, a certain error is introduced owing to the absorption and retention of sugar by the char. Sugars differ markedly in the extent to which they are absorbed by animal charcoal. In the case of the simple reducing sugars, glucose, fructose, etc., the error through absorption is so small as to be almost negligible, but in the case of sucrose and other higher saccharides the absorption is so great that an error of several degrees Ventzke may be occasioned in the polarization.

One method of eliminating the error through absorption of sucrose consists in adding a correction previously established by experiment upon pure sugar solutions. If, for example, a sucrose solution polarizing 95.0° V. gives, after shaking 50 c.c. with 2 gms. of charcoal for 5 minutes, a polarization of only 94.7° V., then a correction of 0.3° V. must be added to all polarizations of about 95° V. for sugars decolorized in this same way. A correction table is thus made for sugar solutions of different concentrations, but in applying these corrections care must be taken that the quality and quantity of the char are alike in both instances and that the time of shaking is always the same. With impure products of variable composition the employment of absorption factors is attended with considerable uncertainty.

Spencer* has recommended a different method of employing animal charcoal for the purpose of reducing the absorption error to a minimum. The process is thus described:

"Place a small quantity of bone black, about 3 gms., in a small plain filter, selecting a rather slow filtering paper. Add a volume of the solution equal to that of the char, or just completely moisten the latter, and let this liquid filter off. After four or five similar filtrations, the filtrates from which are rejected, test the filtrates by a polariscopic observation and note whether the reading varies. Solutions must be protected from evaporation during the filtration. As soon as the reading is constant, showing no further absorption, record it as the required number."

The method just described, while largely eliminating, does not completely remove, the errors of absorption, for while the retention of

* Spencer's "Handbook for Cane Sugar Manufacturers" (4th Ed.), p. 89.

sucrose by the char rapidly diminishes with each successive portion of solution, it soon becomes only a gradually receding quantity. This is shown by the following experiments upon a sucrose solution polarizing 49.9° V.

| Fraction of filtrate. | Polarization. | Absorption error. |
|---|---|---|
| First running | 48.9 | 1.0 |
| Second running | 49.4 | 0.5 |
| Third running | 49.75 | 0.15 |
| Fourth running | 49.80 | 0.10 |
| Fifth running | 49.80 | 0.10 |

With dark-colored solutions it also happens that with each succeeding portion of the filtrate, the charcoal loses its absorptive power for coloring matter as well as for sucrose, so that the final running least free from the error of absorption is too dark for satisfactory polarization.

The general consensus of opinion regarding the use of animal charcoal in sugar analysis is that it should be used as a decolorizing agent only as a last resort. Its employment in the polarization of raw cane sugars has been condemned by the International Commission upon Unification of Methods.* In the polarization of low-grade sugar products its use, however, seems at times justified by necessity; in all such cases efforts should be made to reduce the absorption error to a minimum.

**Decolorization of Sugar Solutions by Means of Hydrosulphites.** — Attempts have been made to employ various decolorizing agents for the purpose of avoiding the precipitate errors of basic lead salts and the absorption error of bone black. The most promising of the numerous substances which have been tried in this connection are the salts and derivatives of hydrosulphurous acid.†

The employment of commercial hydrosulphite preparations, such as " Blankit," " Redo," etc., has been common in the sugar factory,

* See page 202.

† The dry sodium hydrosulphite is prepared by allowing zinc, sodium bisulphite, and sulphuric acid to react in the following molecular proportions:

$$2\,NaHSO_3 + Zn + H_2SO_4 = ZnS_2O_4 + Na_2SO_4 + 2\,H_2O.$$

The zinc hydrosulphite is then decomposed with sodium carbonate,

$$ZnS_2O_4 + Na_2CO_3 = Na_2S_2O_4 + ZnCO_3.$$

The sodium hydrosulphite is salted out from solution by means of sodium chloride and dehydrated by warming with strong alcohol. The compound is then dried in vacuo at 50° to 60° C.

where they have been used for bleaching dark-colored massecuites and also, in solution, as a wash for whitening sugars in the centrifugal. They have also been employed by unscrupulous manufacturers for bleaching low-grade molasses in the preparation of table sirups.

For their use in sugar analysis the solution to be decolorized is treated with a few cubic centimeters of alumina cream and a few crystals of sodium hydrosulphite (0.1 gm. to 1.0 gm., according to the depth of color); after mixing and dissolving, the volume is made up to the mark, and the solution filtered. The filtrate should be polarized immediately.

In many cases there is a rapid redarkening of solutions decolorized with hydrosulphites. Weisberg,* from his study of the action of hydrosulphites, concludes that the bleaching action is a double one, first, by means of the free sulphurous acid when decolorization is permanent, and secondly by means of the nascent hydrogen which is evolved, when there is a redarkening of the solution through oxidation. Afterdarkening may be prevented by the use of another hydrosulphite derivative, sodium sulphoxylate-formaldehyde, sold commercially as "Rongalite." The latter, however, is much slower in its bleaching action than hydrosulphite and is not always an effective decolorizing agent.

A serious objection against hydrosulphite is its action upon the polarizing power of certain reducing sugars. Bryan† has found that the polarizing power of glucose was decidedly lowered after the addition of hydrosulphite, owing to the formation of a levorotatory oxysulphonate. Rongalite did not produce this effect. Neither rongalite nor hydrosulphite caused any immediate change in the polarization of fructose or sucrose. Numerous cases of inversion of sucrose by the prolonged action of hydrosulphites have been reported, however, in the literature.

The experience of chemists, in the use of hydrosulphites as a decolorizing agent for sugar analysis, has been upon the whole unfavorable. In many cases the decolorized solution becomes turbid through separation of sulphur, thus rendering polarization impossible. The bleaching action of hydrosulphite is also limited, and has but little decolorizing effect upon caramel substances, which are among the chief causes of discoloration in sugar-house products.

**Aluminum Hydroxide as a Clarifying Agent.** — A common preparation, used in connection with other clarifying agents, yet having but

* Centrbl. Zuckerind, **15**, 975.

† Bull. 116, U. S. Bur. of Chem., p. 76.

little decolorizing power in itself, is aluminum hydroxide, or, as it is more generally termed, "alumina cream." The method of preparing alumina cream, as prescribed by the Association of Official Agricultural Chemists, is as follows:*

"Prepare a cold saturated solution of alum in water and divide into two unequal portions. Add a slight excess of ammonium hydroxide to the larger portion and then add by degrees the remaining alum solution until a faintly acid reaction is secured."

The reagent as above prepared consists of aluminum hydroxide suspended in a solution of ammonium and potassium sulphates. The salts have a certain advantage, when alumina cream is used as an adjunct with lead salts, in helping to precipitate any excess of lead from solution. In certain cases, however, the presence of ammonium and potassium sulphates is detrimental, so that for many purposes it is better to employ a salt-free cream. For the preparation of the latter, concentrated alum solution is precipitated with a slight excess of ammonia and then washed by decantation with water until the solution is free from sulphates. The excess of water is then poured off and the residual cream stored in a stoppered bottle.

The clarifying effect of alumina cream is chiefly mechanical; its action consists largely in carrying down finely suspended or colloidal impurities which would otherwise escape filtration. When used in connection with lead subacetate it promotes the coagulation of the precipitated impurities and renders filtration more perfect and rapid.

For the polarization of very high grade sugars, sirups, honeys, etc., alumina cream is the only clarifying agent required. In all such cases only the salt-free reagent should be used. About 2 c.c. of the cream are sufficient for clarification and the volume of aluminum hydroxide in this amount is too insignificant to affect the polarization.

Concentrated alum solution is sometimes used with lead subacetate for clarifying. The precipitate, formed between the lead salt and alum, helps to remove coloring matter, but the increase in precipitate and other errors tend to nullify any advantages of the method.

*Comparisons of Different Clarifying Agents.*

A few examples, taken from the reports of Referees upon Sugar for the Association of Official Agricultural Chemists, are given in order to show the probable error of different clarifying agents in polarization.

* Methods of Analysis A. O. A. C. Bull. 107 (revised), U. S. Bur. of Chem., p. 40.

TABLE XLII

*Polarization of Mixtures of Sucrose, Glucose, and Fructose with 0.5 gm. Ammonium Oxalate and 0.5 gm. Sodium Sulphate, using Different Clarifying Agents (Bryan)* *

| Clarifying agent. | Amount of clarifying agent used. | Direct polarization. |
|---|---|---|
| Alumina cream | 5 c.c. | 89.00° V. |
| Lead subacetate solution | 3.5 c.c. | 89.50 |
| Lead subacetate solution | 7 c.c. | 89.55 |
| Neutral lead acetate solution | 3 c.c. | 89.20 |
| Neutral lead acetate solution | 6 c.c. | 89.20 |
| Basic lead nitrate solution | 4 c.c. | 89.00 |
| Dry lead subacetate | 1.5 gms. | 89.05 |
| Sodium hydrosulphite | 1 gm. | 88.60 |

Taking the experiment with alumina cream as the true polarization, it is seen that the lead subacetate solution gives a reading 0.5° V. too high and the normal lead acetate 0.2° V. too high. The excess reading in the second case is due to the volume of precipitate and in the former to both volume of precipitate and precipitation of fructose. The dry lead subacetate and basic lead nitrate clarifications give readings practically identical with the true polarization. This might seem to indicate no precipitation of optically active reducing sugars; such a precipitation does take place, however, and the experiment only shows that in this particular instance the various errors of clarification happen to neutralize one another. Treatment with hydrosulphite gives a polarization below the true value owing to the change in rotation of the glucose.

TABLE XLIII

*Polarizations of Raw Cane Sugar and Cane Molasses, using Different Clarifying Agents (Average Results of Several Collaborators)*

| Clarifying agent. | Direct polarization. | |
|---|---|---|
| | Sugar. | Molasses. |
| Alumina cream and hydrosulphite | +92.75 | +41.99 |
| Neutral lead acetate solution | 92.92 | 42.46 |
| Basic lead acetate solution | 93.05 | 42.82 |
| Basic lead nitrate solution | 92.98 | 43.23 |
| Dry lead subacetate | 92.90 | 42.63 |

The experiments show a lower polarization using hydrosulphite, a result due in large part to the change in rotation of glucose. Basic lead

* Bull. 116, U. S. Bur. of Chem., p. 71.

acetate and nitrate solutions give much higher polarizations owing to both the volume of precipitate error and the precipitation of fructose. Neutral lead acetate solution and dry lead subacetate give polarizations between these two extremes, there being, however, in case of the former, a volume of precipitate error and in case of the dry lead an error due to precipitation of reducing sugars. The true polarization would be somewhere between the results obtained with hydrosulphite and neutral lead acetate.

The selection of an appropriate clarifying agent is one of the most important operations of saccharimetry, and in making his selection the chemist must be governed by the requirements of each particular case. Rapid filtration and brightness of clarification are factors which must be considered as well as minimum degree of error. Beginning with products of highest purity alumina cream alone should be used wherever possible. With products of slight discoloration, when alumina cream is insufficient, neutral lead acetate solution should be tried. When alumina cream and neutral lead solution fail, lead subacetate, or basic lead nitrate, or neutral lead acetate with hypochlorite may be employed; dry lead subacetate will usually give more accurate results with sugar-cane and other products containing fructose. Animal charcoal or hydrosulphites should be used only as a last resort, when other means of clarification have failed. The smallest possible quantity of clarifying agent should be used in all cases.

### Polarization of Sugar Products Containing Insoluble Matter

In the analysis of juices, sirups, molasses, massecuites, and sugars, the chemist has to deal with substances which are entirely soluble in water. The work of polarization becomes more complicated when considerable insoluble matter is present, as happens in the analysis of fruits, tubers, stalks, and other vegetable substances or in the examination of filter-press cake, scums, and other sugar-house residues.

The methods for polarization of succulent plant materials may be divided into three general classes: (1) Methods of Expression; (2) Methods of Extraction, and (3) Methods of Digestion. As an illustration of these several methods the polarization of sugar beets offers a good and classic example.

**Sampling Sugar Beets, Etc.** — In preparing sugar-beets, sugar cane, fruits, etc., for analysis the material must first be reduced to a finely divided condition. For this purpose any of the numerous mechanical rasps, shredders, graters, etc., may be employed, provided that the cellular tissue be thoroughly disintegrated and that no losses occur through leakage of juice or evaporation.

*Keil's Beet Sampler.* — The Keil boring machine (Fig. 128) is very frequently used for taking samples of individual sugar beets. The essential feature of the apparatus consists of a hollow detachable bit, the construction of which is shown in Fig. 129. The conical rasp at

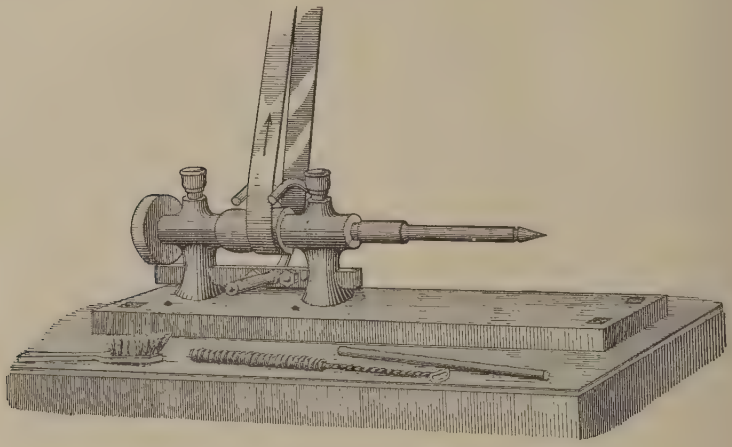


Fig. 128. — Keil's boring rasp for sampling sugar beets.

the end, revolving at a speed of about 3000 revolutions per minute, reduces the substance of the beet to an extreme degree of fineness and at the same time forces the pulp through a small opening into the cavity

Fig. 129. — Detachable bit of Keil's boring rasp.

within. Each beet is bored in an inclined direction, as shown in Fig. 130, in order to secure the best representative sample. When only single beets are examined (as in the selection of "mother beets" for seed production) the bit is detached after each boring and a new one screwed on. The bits are numbered, and to obtain the sample the conical rasp is removed and the pulp (from 8 to 14 gms., according to the size of beet and length of boring) forced out with a rod. In sampling large numbers of beets the bit is kept in constant use, the pulp

being discharged in a continuous stream into a covered container at the end of the apparatus.

*I. Determination of Sugar in Sugar Beets by Expression of Juice*

The determination of the sugar in sugar beets by polarization of the expressed juice was formerly quite common, but has now given place to more accurate methods of analysis.

Fig. 130. — Showing direction of boring in sampling sugar beets.

Assuming (as is incorrect) that the sugar, amides, albuminoids, salts, gums, and other water-soluble solids of the beet are in the same condition of solution within the beet as in the expressed juice, and letting $M$ = the per cent of water-insoluble matter or "marc" and $100 - M$ = the per cent of juice, then the sugar content ($S$) of the beet can be calculated from the polarization ($P$) of the expressed juice by the formula

$$S = \frac{P(100 - M)}{100}.$$

*Example.* — The expressed juice of a sugar beet gave a polarization of 16.2° V. for the normal weight: the beet contained 4.6 per cent of marc. Required the per cent of sugar in the beet.

$$S = \frac{16.2\ (100 - 4.6)}{100} = 15.45 \text{ per cent.}$$

The above method is, of course, equally applicable to the analysis of sugar cane, fruits, and other succulent plant substances.

**Method of Expressing Juice.** — For expressing the juice from the pulp of sugar beets, sugar cane, etc., any suitable form of hand press may be used. The small hydraulic press shown in Fig. 131 is one of great efficiency and is a piece of apparatus almost indispensable in a sugar laboratory.

The pulp to be pressed is placed in a strong sack inside the perforated container $C$, and covered evenly with a heavy metal disk. By turning the wheel $W$ the screw $A$ is driven downward as far as possible upon the disk, thus squeezing out through the openings of $C$ a considerable part of the juice, which escapes by the spout $D$ into a can or other receptacle. The horizontal hydraulic screw $B$ is then turned inwards. This screw, operating by means of glycerol which fills the hollow base $H$, forces the piston $E$ upwards and removes by vertical pressure a second fraction of juice. The final pressure, indicated by

the manometer $M$, can be raised to 300 atmospheres. The juice, as the pressure increases, is of gradually diminishing purity; it is important therefore that all the runnings should be well mixed before taking the sample for polarization.

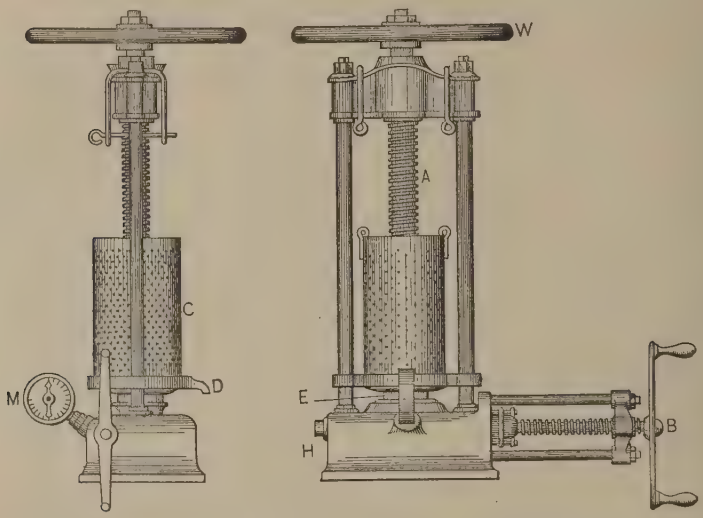



Fig. 131. — Laboratory hydraulic press for expressing juices.

*Determination of Marc.* — A determination of the insoluble cellular matter, or marc, is necessary before the per cent of sugar in plant substances can be calculated from the polarization of the expressed juice. For rough purposes of estimation a constant percentage of 5 per cent or 4.75 per cent marc is sometimes assumed for the sugar beet and 10 per cent or 12 per cent for the sugar cane. Such figures, however, have no exact value, as the percentage of cellular matter varies considerably according to the age of the plant, dryness of the season, and many other conditions.

For the determination of marc 20 to 50 gms. of the finely divided pulp are digested with 200 to 500 c.c. of cold water for 30 minutes, and then filtered as dry as possible upon a piece of finely woven linen, using suction. The washing is repeated with successive portions of cold water until the filtrate, from color and taste, is judged to be free of extractive matter. The residue is then washed several times with hot distilled water, then, after pressing together, with 2 to 3 portions of

90 per cent alcohol, and finally with a little ether. After the ether has volatilized the marc is dried in an oven, gradually raising the temperature after a few hours to between 100° and 110° C. After cooling in a desiccator the residue, which is very hygroscopic, is rapidly weighed (preferably in a stoppered weighing bottle) and the weight taken as the amount of cellular matter or marc. For a determination of the organic cellular matter, the marc is incinerated and the percentage of ash deducted.

The percentage of marc subtracted from 100 gives the percentage of juice.

Where many determinations of marc have to be performed, a battery of small continuously operating percolators will effect a considerable saving of time.

**Errors of Expression Method.** — Several sources of error are involved in the determination of sugar in plant substances by analysis of the expressed juice. In the first place a considerable amount of juice, varying from 10 per cent to 30 per cent, according to the efficiency of the press, is not eliminated and this residual juice, containing a larger amount of albuminoids, pectin, etc., is of much lower purity than the part first expressed. This excess of impurities in the unexpressed juice is washed out, however, in the marc determination. The polarization of the expressed juice is thus higher than that of the composite juice of the entire plant. (See under Distribution of Water, page 230.)

A second source of error is the extraction during the marc determination — by the excessive amounts of cold water, but more especially by the hot water, alcohol, and ether — of variable amounts of hemicelluloses, wax, oil, and other substances which are, strictly speaking, not juice constituents and should therefore be included in the marc. The percentage of juice is thus estimated too high, and a plus error introduced in the calculation. Except for the disadvantage of loss of time in drying, the use of alcohol and ether as dehydrating agents should be omitted in the marc determination, and cold water alone be used for extracting.

*" Colloidal " or " Imbibition " Water.* — A third source of error to be mentioned is the much-debated question of " colloidal " or " imbibition " water, by which is meant water, in a more or less hydrated form, in combination with hemicelluloses and other plant constituents. This imbibed water contains no sugar in solution, and, being expelled from the pulp upon drying, the percentage of sugar-containing juice is overestimated.

Heintz* showed, in 1874, when the air-dried and sugar-free marc of beets was placed in sugar solutions, that water was imbibed, thus leaving the sugar more concentrated and increasing the polarization. In the following experiments by Heintz air-dried beet marc, which had been washed completely free from sucrose, was treated 16 hours in a cool place with solutions containing a normal and half-normal weight of sucrose, in the proportion of 1 gm. marc to 20 c.c. of solution.

| | Half normal weight. | Normal weight |
|---|---|---|
| Polarization before marc treatment | 49.8 | 99.6 |
| Polarization after marc treatment | 53.9 | 104.6 |

The observations of Heintz were verified in a different way by Scheibler.† The latter found that samples of sugar beets, whose expressed juice polarized 14.5 had a marc content of 4.71 per cent. The percentage of sugar in the beets according to the formula

$$S = \frac{P(100 - M)}{100}$$

would be 13.82. Scheibler found, however, by his method of alcoholic extraction a percentage of only 13.1 or a difference of 0.72 per cent. The percentage of sugar-containing juice in the beets, assuming that this juice is of the same polarization as the part expressed, is found by the formula, per cent juice $= 100\frac{p}{P} = 100\,\frac{13.1}{14.5} = 90.34$ per cent. in which $p$ is the polarization of the beets by the extraction method and $P$ the polarization of the expressed juice. The percentages of juice and marc being respectively 90.34 and 4.71, there is left a remainder of 4.95 per cent, which Scheibler termed " colloidal " water. This method of estimation is based, however, upon the assumption that the juice expressed is of the same composition as the combined juices of the beet, which is not exactly true.‡

**Distribution of Water in Plant Tissues.** — The distribution of the water in plant tissues has such an important bearing upon certain problems of sugar analysis that a short discussion of the question may be introduced with profit at this point.

* Z. analyt. Chem. (1874), 262.

† Ibid. (1879), 176, 256.

‡ For a very full discussion with bibliography of the subject of "colloidal" water see Rümpler, " Die Nichtzuckerstoffe der Rüben " (1898), pp. 1–13.

Fig. 132 shows a magnified cross section of a part of a sugar-cane stalk. The sugar-containing juice proper, represented by *S* (the vacuoles), constitutes the principal part of the cell contents in the thin-walled parenchyma or fundamental tissue, and includes the greatest part of the water in the cane. Lining the walls and permeating

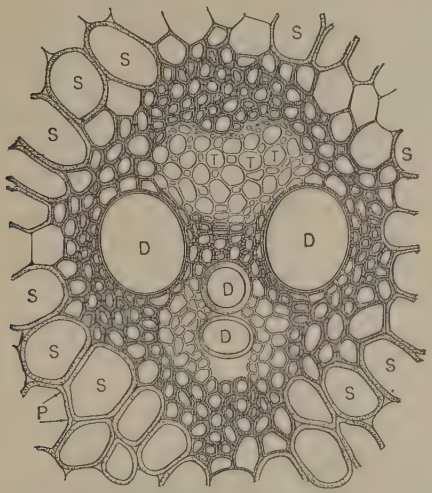



Fig. 132. — Magnified cross-section of sugar-cane (protoplasmic lining P much intensified).

through these cells are thin layers and threads of protoplasmic matter *P* which contains a considerable amount of water, but is deficient in sugar. Running longitudinally through the stalk are large numbers of fibrovascular bundles whose ducts, *D*, are filled with water taken up from the soil. The water of these ducts may often be seen spurting from the end of a cane stalk as it passes between the rollers of a mill, and is found upon analysis to be almost free of sugar. Running parallel with the ducts are the sieve tubes *T* which carry in solution the products of assimilation from the leaf to the stalk. The water of these tubes contains reducing sugars but is deficient in sucrose. The cellular walls of the parenchyma and fibrovascular tissues contain about 50 per cent cellulose, 20 per cent xylan, 5 per cent araban and a remainder of lignin substances, all of which may hold a certain amount of water in the imbibed or colloidal form.

*Variation in Composition of Juice from Different Mills.* — The pressings from the first rollers or crusher of a cane mill consist mostly of the sugar-containing juice *S* (Fig. 132). The pressings from succeeding rollers, where the pressure is greater, contain more and more of the protoplasmic juice *P* and the juice from the ducts and tubes. The colloidal water of the cellular substance is of course not affected by the milling.

The composition of the pressings from the different rollers of a cane mill is given in Table XLIV.

TABLE XLIV

| | First rollers. | Second rollers. | Third rollers. |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| Water | 84.64 | 85.40 | 85.35 |
| Sucrose | 12.93 | 11.41 | 11.30 |
| Reducing sugars | 1.54 | 1.29 | 1.23 |
| Ash | 0.37 | 0.58 | 0.77 |
| Albuminoids | 0.18 | 0.50 | 0.58 |
| Gums, acids, etc | 0.34 | 0.82 | 0.77 |
| Total | 100.00 | 100.00 | 100.00 |
| Per cent extraction of cane | 64.50 | 5.50 | 2.13 |

The pressed cane (bagasse) from the third rollers still contained over 60 per cent of water, corresponding to about 20 per cent of the total juice in the cane. If this residual juice could all be squeezed out by some inconceivable pressure, its sugar content would be much inferior to that of the pressings from the third rollers. It would of course be inaccurate to estimate the sugar content of the cane from the polarization of the first pressings; the same is also true, but to a much less degree, of the composite pressings of several mills.

The impossibility of obtaining by pressure a true composite sample of the different juices of a plant, the difficulty of estimating the true content of marc, and the uncertain influence of the colloidal or imbibed water are the chief objections to the expression methods of sugar determination.

*II. Determination of Sugar in Sugar Beets by Extraction with Alcohol*

The method most accurate in principle for determining sugar in beets and other plant substances, is that of extraction. In this process the sugar is washed out from the pulp and the extract made up to volume and polarized. The errors due to uneven composition of

juices, faulty marc estimation, and colloidal water are thus completely eliminated.

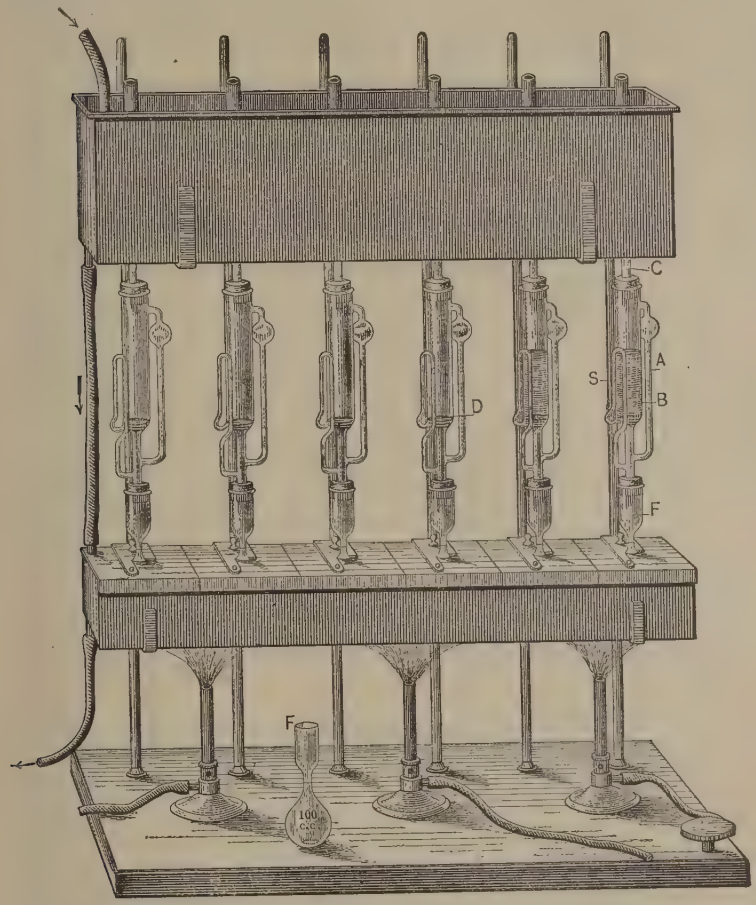



Fig. 133. — Apparatus for Scheibler's alcohol-extraction method.

**Scheibler's Alcohol-extraction Method.** — The solvent most generally used for the extraction of sugar from beet pulp is 90 per cent ethyl alcohol. The original method of Scheibler* as modified by Sickel† is as follows:

* Neue Zeitschrift, **2**, 1, 17, 287; **3**, 242.

† Ibid. **2**, 692.

A normal (or double normal) weight of finely prepared pulp is weighed rapidly in a weighing dish, 3 c.c. of lead subacetate (6 c.c. for the double normal weight) are then added and thoroughly mixed with the pulp by means of a glass rod, adding at the same time 5 to 10 c.c. of 90 per cent alcohol. The pulp is then transferred to the extraction cylinder *B* of a Soxhlet extractor, of which Fig. 133 shows six in the form of a battery. The bottom of the extraction cylinder is covered with a clean wad *D* of felt or cotton; the pulp is washed in with 90 per cent alcohol, and pressed down so that its upper surface is below the upper bend of the siphon tube *S*. The top of the extraction vessel is then connected by means of a tight-fitting cork with the condensing tube *C*, and the bottom with the 100 c.c. flask *F*, which should contain about 75 c.c. of 90 per cent alcohol.

The water in the bath is heated until the alcohol in the flask begins to boil vigorously, when the heat is regulated to this constant temperature. The vapor from the boiling alcohol passes upward through the side tube *A* and condensing in *C* drops back upon the pulp in *B*. As soon as the level of alcohol in *B* rises above the bend of the tube *S*, the alcoholic solution of sugar siphons mechanically into the flask *F*. The distilling and siphoning are continued until all the sugar is extracted, which, according to the fineness of the pulp, usually requires from 1 to 2 hours. Immediately after the last siphoning the flask *F* is disconnected, cooled to room temperature, the volume completed to 100 c.c., and the solution mixed, filtered, and polarized.

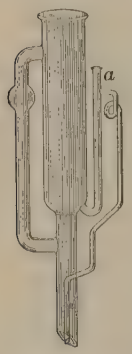


Fig. 134. — Müller's modification of Soxhlet's extractor.

A form of extraction vessel devised by Müller (Fig. 134) permits the withdrawal of a small sample of liquid from the siphon tube for determining the completion of extraction. The opening at *a* is closed during operation with a stopper. To obtain the sample this stopper is removed, a few cubic centimeters of liquid are sucked up with a pipette and subjected to the $\alpha$-naphthol test (page 341). If the test is positive, the stopper is replaced and the extraction continued until the reagent gives no coloration.

In determining sugar by the Scheibler process of extraction special care must be exercised to prevent evaporation of alcohol during filtration. The funnel should be covered with a watch glass and the filtrate received in a cylinder or flask with narrow neck. The first 20 to 30 c.c. of the runnings should be discarded. The greater susceptibility of alcoholic sugar solutions to

expansion and contraction with changes in heat and cold necessitates the maintenance of uniform temperature conditions during the polarization. The specific rotation of sucrose in ethyl alcohol is slightly higher (0.1 degree to 0.2 degree) than in water; but the difference is so small that it falls within the limits of experimental error.

The method of alcoholic extraction gives results considerably lower than those calculated from the polarization of the expressed juice. The results of Scheibler previously quoted (page 230) show a difference of about 0.75 for the polarization of sugar beets.

Some authorities prefer adding the lead subacetate to the alcoholic extract rather than to the pulp previous to extraction. This practice is attended, however, with some danger. One main object of adding the basic lead to the pulp is to neutralize any free acid which would otherwise invert some of the sucrose in the hot solution. In presence of alcohol, lead subacetate solution must be used in lowest possible amount owing to the danger of precipitating sucrose or of changing its specific rotation through formation of lead saccharate.

The alcoholic extraction method can be applied to the polarization of fruits and all other sugar-containing plant substances. With very dry materials the strength of the alcohol should be correspondingly reduced. With substances containing reducing sugars in large amount, it is desirable to omit the addition of lead subacetate, but when this is done the substance should be well mixed with powdered calcium carbonate to neutralize any free acid that might cause inversion.

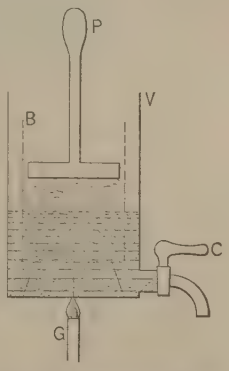


Fig. 135. — Section of Zamaron's hot-water extraction apparatus.

### *II. Determination of Sugar in Plant Substances by Extraction with Water*

Water is sometimes used instead of alcohol in extracting sugar for the polarization of plant substances. In such cases a process of percolation must be used in place of distillation owing to the danger of decomposition through the prolonged boiling of aqueous extracts. As an example of the water extraction process the Zamaron* method for determining sugar in sugar cane is given.

**Zamaron's Water-extraction Apparatus.** — The Zamaron extraction apparatus (Figs. 135 and 136) consists of a cylindrical copper vessel

* Sidersky's "Manuel," p. 261.

$V$ provided at the bottom with a small cock $C$. A basket $B$ of perforated copper, provided with a tripod support, fits loosely within this copper vessel; 100 gms. of the finely divided pulp are transferred to the basket, and 200 c.c. of hot water poured in, the pulp being pressed

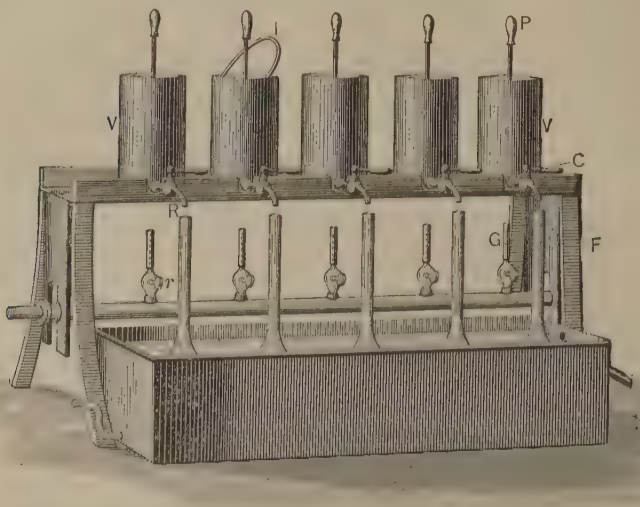



Fig. 136. — Battery of Zamaron's hot-water extractors.

down beneath the surface of the liquid by means of the plunger $P$. The contents of the vessel are then boiled for 10 minutes, after which the flame is turned down, the cock opened, and the hot solution drawn off into the 1000-c.c. graduated flask $F$, as much as possible of the liquid being pressed out by means of the plunger. The cock is then closed and the process repeated with a second portion of 150 c.c. water. The process is continued 6 times, making altogether about 950 to 975 c.c. of extract. After cooling and adding a few cubic centimeters of lead subacetate, the contents of the flask are made to 1000 c.c., shaken, filtered, and polarized in a 400-mm. tube. The reading multiplied by 1.3 gives the polarization (degrees Ventzke) of the sugar cane.

The principal objection, which has been brought against the Zamaron process, is the danger of incomplete extraction. Some idea of the probable magnitude of this error may be formed from the following consideration:

Suppose a sugar cane to contain 18 per cent of sucrose; suppose also that 6 extractions of the pulp are made and that one-third of the liquid is retained by the fiber after each extraction. If the sugar is evenly diffused through all parts of the liquid at the end of each 10 minutes boiling, as is no doubt very nearly true, there would be the following percentages of sugar removed at each extraction.

| | Percentage removed of total sugar. | Percentage of sugar removed per 100 of cane. |
|---|---|---|
| First extraction | 66.66 | 12.00 |
| Second extraction | 22.22 | 4.00 |
| Third extraction | 7.41 | 1.33 |
| Fourth extraction | 2.47 | 0.44 |
| Fifth extraction | 0.82 | 0.15 |
| Sixth extraction | 0.27 | 0.05 |
| Amount extracted | 99.85 | 17.97 |
| Amount unextracted | 0.15 | 0.03 |

It is seen that the residual sugar left after 6 extractions can be only very slight. In order to reduce the possibility of error through incomplete extraction Fribourg* recommends that only 50 gms. of pulp be taken for analysis. This, however, while halving the errors of extraction, necessitates a doubling of any error in the polariscope reading.

Another source of error, in the method of hot water extraction as described, is the danger of inversion of sucrose through the natural acidity of the pulp. One method of preventing this is to mix with the pulp previous to extraction finely powdered calcium carbonate. Another method* is to employ very dilute milk of lime water for the extraction. The presence of minute quantities of free alkali does not affect the determination of sucrose; a danger exists, however, in the action of hot alkaline solutions (even where very dilute) in modifying or destroying reducing sugars. Careful neutralization of the free acid in the pulp with lime water, or dilute sodium hydroxide, would eliminate the risk of inversion without serious danger of affecting the reducing sugars.

Another objection to the method of hot-water extraction is the solution of optically active dextrins, gums, and hemicelluloses. These substances introduce at times a considerable error in the polarimetric determination of sugars in aqueous plant extracts. The error does

* Fribourg's "Analyse chimique," p. 223.

not exist in the alcohol-extraction method, owing to the insolubility of dextrinoid substances in ethyl alcohol.

*III. Determination of Sugar in Sugar Beets by Methods of Digestion*

The method of alcoholic extraction, although the most accurate and scientifically perfect, is not the best from a practical standpoint on account of the long period of time necessary for extraction, and also because of the rather fragile nature of the extraction apparatus. For the rapid determination of sucrose in sugar beets some one of the numerous digestion processes is usually followed.

The digestion method may be regarded in principle as a combination of the extraction and juice-expression methods. A weighed amount of pulp is digested with 5 to 6 times its volume of alcohol or water. After the complete diffusion of the sugar through the liquid, the solution is made up to volume, allowing for the space occupied by insoluble matter, and then filtered and polarized.

**Rapp-Degener Alcohol-digestion Method.** — The first process of digestion employed alcohol, and is known as the Rapp-Degener* method. The double normal weight of fine beet pulp is transferred to a 201.2-c.c. flask (the extra 1.2 c.c. being the estimated volume of the insoluble cellular matter in 52. gms. of pulp). The forms of flask shown in Fig. 137 are convenient for the purpose. Three to four c.c. of lead-subacetate solution are mixed with the pulp and then about 150 c.c. of 90 per cent alcohol added. The flask is closed with a stopper containing a condensing tube and placed in a hot-water bath. The alcohol is gently boiled for 20 minutes, when diffusion of the sugar through the solution may be considered complete. The tube and stopper are rinsed into the flask and the volume completed nearly to the mark with 90 per cent alcohol. The flask is again placed in the hot-water bath for 1 to 2 minutes, to secure even mixing of the contents and expulsion of air bubbles, and then allowed to cool slowly in the air for $\frac{1}{2}$ hour. The liquid is then brought to room temperature and the volume completed to 201.2 c.c. with 90 per cent alcohol. The solution is then mixed, filtered and polarized in a 200-mm. tube, using the necessary precautions to prevent evaporation and changes in temperature.

Fig. 137. — Flasks for alcoholic digestion of beet pulp.

* Z. Ver. Deut. Zuckerind., **32,** 514, 786.

The employment of alcohol in analytical work is expensive; it was also found that with any coarse particles of pulp the diffusion of sugar through the alcohol was considerably retarded. Pellet* was accordingly induced in 1887 to devise a method for determining sugar in beets in which water was used for digesting instead of alcohol. The Pellet method may be carried out with either hot or cold water.

**Pellet's Cold-water-digestion Process.**—Twenty six gms. of finely divided pulp are transferred by means of a jet of water into a 200.6-c.c. flask (the extra 0.6 c.c. being the estimated volume of the insoluble marc in 26 gms. of pulp); 5 to 6 c.c. of lead-subacetate solution are then added and sufficient water to fill the flask about two-thirds. After mixing, the flask is allowed to stand for 20 to 30 minutes to permit

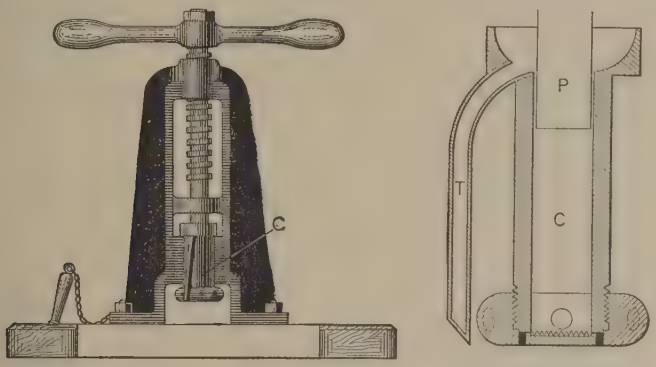



Fig. 138. — "Sans-Pareille" press for preparing finely divided pulp. The substance, which is placed in the cell *C*, is forced in a semiliquid condition by the piston *P* through the fine openings at the bottom into a container underneath; the latter also receives any overflow of juice which escapes by the outlet *T*.

diffusion of sugar and allow enclosed air bubbles to escape. Water is then added nearly to the mark, any foam destroyed with a drop of ether, and the volume completed to 200.6 c.c. The solution is well mixed, filtered, and polarized in a 400-mm. tube; the scale reading gives without correction the polarization of the beet.

With pulp of extreme fineness, such as is obtained with the "Sans-Pareille" press (Fig. 138), the diffusion of sugar from pulp to water becomes almost instantaneous, and the solution can be completed to volume as soon as air bubbles have arisen. The time of analysis is thus considerably lessened.

* Deut. Zuckerind. (1888), 1229; (1889), 531.

**Pellet's Hot-water-digestion Process.** — If apparatus is not available for obtaining pulp of suitable fineness, hot water should be used to promote the diffusion of sugar from the coarser particles of pulp. Twenty-six grams of pulp, mixed with 6 c.c. of lead-subacetate solution, are washed into a 200.6 c.c. flask, water is added with shaking until the volume is almost up to the mark, and the flask heated in a boiling water bath for $\frac{1}{2}$ to 1 hour, according to the fineness of the pulp. The flask is then immersed in cold water; as soon as the contents are of room temperature, the volume is completed to the mark. The remainder of the process follows as under cold-water digestion.

**Krüger's Cold-water-digestion Process.** — Krüger,* in 1896, devised a water-digestion process, an interesting feature of which is that the use of normal weights and of volumetric flasks is entirely dispensed with. The principle of the method may be understood from the following:

The weight of juice per 26 gms. in an average sugar beet of 5 per cent marc content is $26 \times 0.95 = 24.7$ gms. The specific gravity of the average beet juice is very nearly 1.07, so that the volume of juice in a normal weight (26 gms.) of pulp is 24.7 gms. $\div$ 1.07 = 23.08 c.c. The amount of water necessary to complete this volume of juice to 100 c.c. is therefore $100 - 23.08 = 76.92$ c.c. The ratio of normal weight to volume of added water is then 26 gms. : 76.92 c.c. = 1 gm. : 2.96 c.c., or in round numbers 1 gm. : 3 c.c. The addition, therefore, of water in the proportion of 3 c.c. to every 1 gm. of pulp yields a solution whose polarization in a 200-mm. tube will give the approximate sugar content of the beet.

The automatic pipette (Figs. 139, 140) for rapidly measuring water and lead solution is an essential feature of the Krüger process. The pipette is prepared in several sizes for approximate double-normal, normal, half-normal, and quarter-normal weights of pulp (i.e., approximately 50, 25, 12, and 6 gms.), the smaller sizes being used in polarizing mother beets, where the quantities of pulp obtained by the Keil sampler (p. 226) are small (8 to 14 gms.). The pipette, which is fastened to a fixed support $S$ (Fig. 140), is provided at opposite ends with the three-way cocks $C$ and $C'$, the movements of which are controlled by the double lever $L$. The lower inlet of the pipette is connected by the tube $A$ to the vessel $V$ which contains the "lead water" (9 vols. of water to 1 vol. of lead-subacetate solution). The upper outlet which permits the escape of air is connected with the upright tube $B$. By raising $L$ to the stop $c$ (Fig. 139) the pipette is filled with "lead water,"

* Deut. Zuckerind. (1896), 2434.

any overflow passing into the tube *B*. Upon dropping *L* to the stop *d*, the cocks are both reversed, air entering through *f*, and the contents of the pipette being discharged through *e* into the metal weighing dish *D*, which contains the weighed sample of pulp.

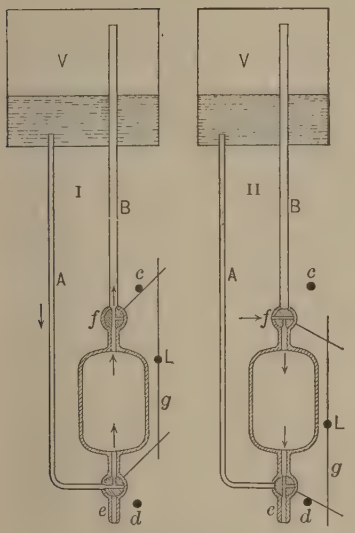



Fig. 139

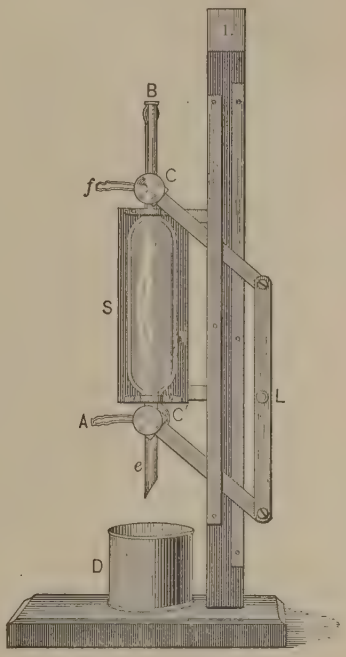



Fig. 140

Krüger's automatic pipette for sugar beet analysis.

The weight of pulp corresponding to each pipette is determined by calibration with water, as in the following example. The weight of distilled water discharged by a Krüger pipette at 20° C. was found to be 78.38 gms. The volume of the pipette in true cubic centimeters is then $78.38 \div 0.9972 = 78.6$ c.c. $78.6 \div 3 = 26.2$ gms., the weight of beet pulp corresponding to the pipette.

After mixing the pulp and "lead water" the weighing dish is covered and the contents allowed to remain for 20 to 30 minutes. The solution is then well stirred, filtered, and polarized in a 200-mm. tube.

The Krüger method, while not claiming extreme accuracy, is sufficiently exact for many purposes of analysis. On account of its simplicity and rapidity the method has been widely used in such places as beet-seed nurseries, depots for purchase of beets, etc., where large numbers of samples have to be polarized with the least possible loss of time.

**Sachs-Le Docte Process of Water Digestion.** — The occlusion of air bubbles by pulp and the uncertainty of knowing whether such bubbles are completely absent before making up to volume have been the principal objections against the original Pellet process of digestion. This error does not occur in the Krüger method, where the volume of solution is established independent of any occluded air. The necessity of employing irregular weights for each individual pipette and the use of insufficient water for the complete diffusion of the sugar during the cold digestion have been raised on the other hand as objections against the Krüger method. Sachs * and Le Docte † have met these difficulties by always taking the regular normal weight (26 gms.) of pulp for analysis and adding a constant volume (177 c.c.) of water and lead subacetate so that the final estimated volume of solution, regardless of insoluble marc or occluded air, is always 200 c.c.

The constant-volume figure 177 c.c. in the Sachs-Le Docte process is derived from the following consideration. Sachs assumes as the average marc and juice content of the sugar beet 4.75 per cent and 95.25 per cent respectively. For the normal weight (26 gms.) of pulp there would then be 26 gms. $\times$ .9525 = 24.765 gms. juice. The average sugar content and density of juices from beets of different richness are given in the following table together with the calculated volume of juice (24.765 $\div$ sp. gr.), the volume of lead-water solution (200 c.c. less the volume of juice) and the polarization error resulting from use of the constant volume 177 c.c.

TABLE XLV

| Sugar in beet. | Sugar in juice. | Brix of juice. | Specific gravity of juice. | Volume of juice. | Volume of lead-water solution. | Calculated polarization.* | †Polarization error. |
|---|---|---|---|---|---|---|---|
| Per cent. | Per cent. | | | c.c. | | | |
| 12 | 12.59 | 14.86 | 1.0609 | 23.34 | 176.66 | 11.979 | −0.021 |
| 13 | 13.65 | 15.82 | 1.0651 | 23.25 | 176.75 | 12.984 | −0.016 |
| 14 | 14.70 | 16.82 | 1.0694 | 23.16 | 176.84 | 13.988 | −0.012 |
| 15 | 15.75 | 17.86 | 1.0740 | 23.06 | 176.94 | 14.995 | −0.005 |
| 16 | 16.80 | 18.92 | 1.0787 | 22.96 | 177.04 | 16.003 | +0.003 |
| 17 | 17.85 | 20.00 | 1.0835 | 22.86 | 177.14 | 17.012 | +0.012 |

* Calculated polarization = $\frac{\text{sugar in beet} \times 200}{\text{volume of juice} + 177}$.

* Z. Ver. Deut. Zuckerind. (1906), **56**, 918. † Ibid. (1906), **56**, 924.

It is seen that by use of the constant volume 177 c.c. the calculated polarization error is too small to be detected upon the saccharimeter.

The constant-volume pipette employed in the Sachs-Le Docte process is shown in Fig. 141. A three-way cock $K$ at the bottom serves for the inlet of lead reagent and water at $B$ and $C$ and for the delivery of the 177 c.c. of mixed solution through $D$. The cap $A$ at the top, which receives the overflow, is connected with a waste bottle. Instead of drawing in the lead reagent and water separately, a single "lead-water" solution of proper dilution may be used. One of the cock connections may thus be dispensed with. By raising or lowering the capillary tube $h$ upon its support at $H$ the capacity of the pipette is easily adjusted to exactly 177 c.c.

The method of operation is similar to that in the Krüger process. Weigh 26 gms. of pulp in one of the tared metal beakers; the latter are of about 250-c.c. capacity and are provided with a tight-fitting cover of rubber; add 177 c.c. of water containing 5 to 6 c.c. of lead subacetate solution (of about 30° Bé.) and shake thoroughly. Filter, add a drop of glacial acetic acid to the filtrate, and polarize in a 400-mm. tube. The scale reading gives the polarization of the beet. Where many analyses have to be performed a large number of metal beakers are used, all of which are counterpoised against the same weight.

If the particles of pulp are coarse the Sachs-Le Docte process should be carried out by hot digestion.*

Fig. 141.—Sachs-Le Docte automatic pipette for sugar beet analysis.

* Sucrerie Belge, Oct. 15, 1908. Bull. assoc. chim. sucr. dist., **27,** 180.

method of operation is similar to that just described, except that the metal beakers, after addition of the 177 c.c. of lead-water solution to the pulp, are each covered with a special pneumatic cap of rubber which prevents any loss by evaporation. Fig. 142 shows a water bath for the Sachs-Le Docte hot-digestion process. The metal beakers are placed for 30 minutes in a water bath heated to 80° C. After cooling the beakers are well shaken, when the contents are filtered and polarized in the usual way.

Herzfeld* has slightly modified the Sachs-Le Docte process for hot digestion. The pulp is weighed into small copper cans, 11 cm. high,

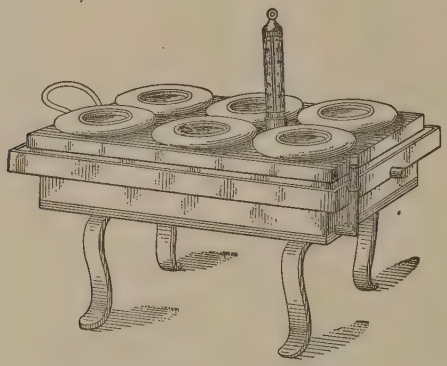


Fig. 142. — Sachs-Le Docte bath for hot-water digestion.

6 cm. body diameter, and 4 cm. mouth diameter. The cans are closed during digestion with rubber stoppers or with good corks covered with tinfoil. The blowing out of stoppers during digestion has been raised as an objection against the Herzfeld modification. Stanek and Urban† recommend the use of cans provided with a spring cap and rubber gasket.‡

A comparison of sugar determinations in beets by the Sachs-Le Docte cold- and hot-digestion methods and by the Krüger method is given in the following table. The results are the average of many determinations reported by Herzfeld.*

* Z. Ver. Deut. Zuckerind., **59,** 627.

† Z. Zuckerind. Böhmen, **34,** 625.

‡ A very full description of methods for analyzing sugar beets and a complete bibliography of the subject from 1839 to 1907 has been compiled by Bryan. (Bull. 146, U. S. Bur. of Chem.)

| | Sachs-Le Docte method. | | Krüger method, cold digestion. |
|---|---|---|---|
| | Cold digestion. | Hot digestion. | |
| | Per cent. | Per cent. | Per cent. |
| Average 14 analyses.... | 16.66 | 16.87 | 16.56 |
| Average 19 analyses.... | 15.91 | 16.28 | 16.12 |

### Errors of Digestion Methods

*Solution of Dextrorotatory Gums.*—It is noted in the preceding table that the hot-digestion gives from 0.2 to 0.3 higher than the cold-digestion methods. This excess is no doubt due in large part to a higher extraction of sucrose from the coarser particles of pulp. Some chemists, however, attribute a part of the excess to a solution of dextrorotatory hemicelluloses (parapectin, metapectin, etc.) which are dissolved by the hot water from the pulp. According to Pellet these substances are completely precipitated by the lead-subacetate solution, when this reagent is of proper strength (about 30 degrees Bé.) and used in proper amount (5 to 6 c.c. per 26 gms. of pulp). To insure complete precipitation of all dextrorotatory gums some authorities advise using 7 or 8 c.c. of basic-lead solution. Herzfeld,* however, has shown that lead subacetate in hot solution forms a levorotatory combination with certain constituents of beet pulp and is opposed to the use of more than 5 c.c. of the reagent per 26 gms. pulp for hot-water digestion.

The extraction of high polarizing dextrorotatory gums is very liable to occur, even with cold-water digestion, in the case of sugar beets which are unripe, frost-bitten, diseased, or otherwise abnormal. Under such circumstances the method of extraction with alcohol, in which the gums are insoluble, should be employed.

*Solution of Asparagine.* — Another constituent of sugar beets which may introduce an error in the polarization is asparagine. Degener† has shown that asparagine, which in neutral solutions is slightly levorotatory ($[\alpha]_D = -5.2$), becomes strongly dextrorotatory ($[\alpha]_D = +61.76$ to $+ 69.10$) in presence of 10 per cent lead-subacetate solution, every 0.1 per cent asparagine polarizing about the same as every 0.1 per cent sucrose. To obviate this error the French chemists add a drop of glacial acetic acid to the filtered solution from the aqueous digestion before polarizing. Asparagine is dissolved only 1 part in 290 parts of

* Z. Ver. Deut. Zuckerind., **59**, 627.

† Deut. Zuckerind. (1897), 65.

80 per cent alcohol and this solubility is diminished by the addition of lead subacetate. The asparagine error is therefore negligible in the methods of alcoholic extraction or digestion.

*Variation in Marc Content.* — Among other sources of error peculiar to the digestion methods may be mentioned the difference in quantity and volume of insoluble cellular matter in the normal weight of pulp. This volume is in fact variously given by different authorities as 0.6 c.c.,* 0.75 c.c.,† 1.35 c.c.,‡ and the digestion flasks have been correspondingly graduated at 200.6 c.c., 200.75 c.c., and 201.35 c.c. Pellet has devised a special digestion flask with 5 graduations at 200.0 c.c., 200.5 c.c., 200.75 c.c., 201.0 c.c., and 201.5 c.c., so that the chemist may vary the volume according to the weight and character of pulp. While the volume most generally prescribed is 200.6 c.c. for 26 gms. of pulp, it is evident that this figure must be greater for wilted beets and less for unripe beets. In the same way the volume of lead-water solution in the Sachs-Le Docte process would be greater or less than 177 c.c. The polarization errors due to normal variations from the average of 4.75 per cent marc are considerably less than 0.1, but in extreme cases of wilted or watery beets the alcoholic extraction method should be used as a control.

The error due to imbibition or colloidal water (p. 229) has also been raised against the digestion methods. The average difference between the expression and extraction methods was found by Scheibler to be about 0.75 per cent, which difference represents the combined influence of unequal composition of juice and of the colloidal water. In the digestion methods the 23 c.c. of juice is diluted to 200 c.c. or nearly ninefold, so that the combined errors of the juice methods are reduced to less than 0.1. In the digestion methods the error due to unequal composition of juice is largely eliminated; the residual error due to the so-called colloidal water must therefore be very small.

The agreement between the aqueous digestion and alcoholic extraction methods upon normal sugar beets is usually very close. As to which of the water-digestion methods is preferable it may be said that if apparatus is available for securing pulp of extreme fineness the cold-water digestion is upon the whole less open to error. But for pulp of coarse or uneven character hot-water digestion should be used to insure complete extraction.

* Frühling's "Anleitung," 209.

† Fribourg's "Analyse chimique," 253.

‡ Sidersky's "Manuel," 241.

### Polarization of Plant Substances Containing but Low Percentages of Sugar

The methods previously described may be applied with minor modifications to the polarization of plant substances containing but low percentages of sugar. The polarization of spent sugar-beet chips and sugar-cane bagasse may serve as illustrations of the methods.

**Polarization of Spent Beet Chips by the Expression Method.** — While the water circulating through the diffusion battery removes most of the sugar from the beet chips, a small amount of sugar always remains unextracted; this residual sugar occurs for the most part within the uncrushed cells of the beet. It is necessary, therefore, in squeezing out the water from diffusion chips to apply extreme pressure, in order to secure the maximum quantity of residual sugar. A polarization of the expressed diffusion water and a determination of its amount are sufficient for the calculation.

*Example.* — 100 c.c. of the diffusion water pressed from a sample of spent beet chips were clarified with 2 c.c. of lead-subacetate solution and the volume completed to 110 c.c. The filtered solution gave a polarization of 2.0° V. in a 400-mm. tube. The water content of the chips, upon drying 10 gms. at 100° to 110° C. to constant weight, was 90.5 per cent.

The polarization corrected for the dilution is $2.0 \times 1.1 = 2.2°$ V. Calling the sp. gr. of the waste diffusion water 1.000 (which can be done without serious error) the polarization of a normal weight would be $(26.00 \times 2.2) \div 100 = 0.572°$ V., or for a 200-mm. tube 0.29° V. The polarization of the spent chips would then be $(90.5 \times 0.29) \div 100 = 0.26$.

**Polarization of Dried Beet Chips by the Alcoholic Digestion and Extraction Method.** — Dried sugar-beet chips have frequently undergone a change in composition through formation of water-soluble optically active gums at the high temperature of drying. The aqueous digestion method may then give a polarization different from the true sucrose content. In such cases it is recommended to use the alcoholic digestion and extraction method of Herzfeld.*

A half normal weight of the finely ground dry chips is digested in a hot-water bath with 50 to 60 c.c. of 60 per cent alcohol, adding 3 to 5 c.c. of lead-subacetate solution, for 30 minutes. The contents of the digestion flask are then transferred by means of a little 60 per cent alcohol to a Soxhlet extractor and extracted under reduced pressure for 5 to 6 hours (see Fig. 143). The alcoholic extract is then made up to 100 c.c., filtered, and polarized in a 400-mm. tube.

* Z. Ver. Deut. Zuckerind., **59**, 627.

**Polarization of Sugar-cane Bagasse by Hot-water Extraction.** — The hot-water-extraction method of Zamaron may be employed upon bagasse in the same manner as described for sugar cane. Owing, however, to the much larger amount of cellular matter in bagasse only 50 gms. are taken for extraction. The extract is made up to 1000 c.c. and polarized in a 400-mm. tube. The reading multiplied by 2.6 gives the polarization of the bagasse.

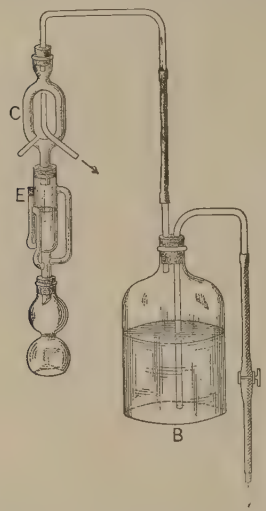



Fig. 143. — Herzfeld's apparatus for alcoholic extraction under reduced pressure.

Extraction waters of very low sugar content are sometimes concentrated before polarization. Five hundred cubic centimeters of the neutralized solution are evaporated to somewhat less than the desired volume, and then made up to 100 c.c. or 250 c.c. for polarization. The saccharimeter reading is divided by 5 or 2 to obtain the polarization of the extract.

**Polarization of Sugar-cane Bagasse by Hot-water Digestion.** — Bagasse is also polarized by the method of hot-water digestion, in which case, however, it is necessary to know the percentage of fiber. The determination may be made by the methods of the Hawaiian chemists.*

*Determination of Fiber in Bagasse.* — One hundred grams of bagasse are placed in a strong linen bag, and the juice pressed out with an hydraulic press. The sample is then treated with cold running water for two minutes, and again pressed, the two operations being repeated alternately five times. The bag is then placed in an air bath at 125° C. for half an hour, after which the fiber is removed from the bag and dried in a shallow dish for four hours at the same temperature. When an hydraulic press is not available, the sample may be treated in cold running water for 12 hours and dried as above described.

*Digestion of Bagasse.* — Fifty grams of bagasse are weighed in a tared flask; 500 c.c. of water containing 2 c.c. of 5 per cent sodium carbonate are added, and the flask connected with a vertical condenser. The solution is boiled gently for one hour, the flask being shaken thoroughly every 15 minutes. After cooling the flask is reweighed, and the weight of contents determined. The weight of contents multi-

* Hawaiian Planters' Record, **3**, 317.

plied by 2 gives the weight ($W$) of fiber and solution corresponding to 100 gms. of bagasse. Letting $F$ = the per cent fiber in the bagasse, $W - F$ = the weight of solution corresponding to 100 gms. of bagasse.

The aqueous extract obtained by the hot digestion is squeezed out; 99 c.c. of the solution are made up to 100 c.c. with lead-subacetate reagent, filtered, and polarized in a 400-mm. tube. The polarization ($P$) corrected for dilution is $\frac{100\,P}{99}$, and this reduced to a normal weight of extract is $\frac{26}{100} \times \frac{100\,P}{99} = \frac{26\,P}{99}$, which value for a 200-mm. tube becomes $\frac{13\,P}{99}$. The polarization of the bagasse is then found by the formula $\frac{13\,P}{99} \times \frac{(W - F)}{100} = \frac{P(W - F)}{7.6 \times 100}$.

## Polarization of Substances Containing Insoluble Mineral Matter

The polarization of substances containing insoluble mineral matter can in general be carried out by the methods of extraction or digestion previously described. Certain classes of products, however, such as carbonatation filter-press cake may contain sugar in the form of insoluble saccharates, and in such cases special methods of treatment are required. As examples of methods to be employed several processes for the polarization of filter-press cake will be described.

**Polarization of Filter-press Cake Free from Saccharate.** — If saccharate-free press cake be triturated with a known quantity of water and the filtered extract polarized, the polarization of the cake may be calculated very closely, provided its moisture content has been determined.

*Example.* — 50 gms. of press cake were ground in a mortar with 200 c.c. of water. The solution (which should not be alkaline) was then clarified with a little dry lead subacetate and polarized in a 400-mm. tube. A reading of 5.2° V. was obtained. The moisture content of the cake, determined by drying 10 gms. in a hot-water bath to constant weight, was 45.6 per cent. It is desired to know the polarization of the cake.

The weight of water in the 50 gms. of cake is $50 \times 0.456 = 22.8$ gms. The total volume of liquid (disregarding the slight increase in volume through solution of sugar) is then $200 + 22.8 = 222.8$ c.c. The polarization of the solution reduced to a normal weight of 26 gms. to 100 c.c. (calling the sp. gr. 1.000, which may be done without serious error) is $(5.2 \times 26) \div 100 = 1.35°$ V., which for a 200-mm. tube is 0.68° V., or 0.68 gms. of sucrose in 100 c.c. of

solution. This corrected to 222.8 c.c. = $0.68 \times 2.228 = 1.52$, the grams of sucrose in 50 gms. of cake; $1.52 \times 2 = 3.04$, the polarization or percentage of sucrose in the cake, if no other optically active substances are present.

The above method of calculation is sufficiently exact for substances of low polarization. When the polarization is high, however, neglect of the increase in volume through solution of sugar and of the change in specific gravity introduces a considerable error. In such cases the polarization should be determined by some method of extraction.

In sugar-house practice the determination of moisture in the press cake is usually dispensed with, it being assumed that the volume of insoluble matter in 26 gms. of cake is 4 c.c. The normal weight of cake is then made up to 104 c.c.; or, if a 100-c.c. flask be used, 25 gms. of cake, when triturated, clarified with lead solution, and the liquid made up to volume, will give the polarization (104 : 26 : : 100 : 25). In practice 50 gms. of cake are generally weighed out and the volume made up to 200 c.c.

In the previous example if the 50 gms. of cake had been made up with water to 200 c.c., there would be 192.3 c.c. of solution (allowing 4 c.c. for volume of insoluble matter in 26 gms.). The polarization for 222.8 c.c. of solution was 5.2° V., therefore 192.3 : 5.2 : : 222.8 : 6.02, the calculated polarization of the cake for a 400-mm. tube. This for a 200-mm. tube would be 3.01, which is only 0.03° V. lower than the result previously found.

**Polarization of Filter-press Cake Containing Saccharate.** — When filter-press cake contains insoluble saccharates, the sugar must be liberated from combination before the solution to be polarized is made up to volume. Several methods have been followed for accomplishing this result.

*Decomposition of Saccharate by Means of Acetic Acid.* — The 50 gms. of press cake, after transferring with water to a 200-c.c. flask, are heated to boiling, and acetic acid added drop by drop until all free alkali is neutralized. The solution is then cooled, clarified, made up to volume, filtered, and polarized as previously described.

*Decomposition of Saccharate by Means of Carbon Dioxide.* — The method is practically the same as that just described, except that a stream of carbon dioxide led into the solution is used for decomposing the saccharate, instead of acetic acid.

The frothing, caused by evolution of carbon dioxide, is the principal objection against the acetic-acid method, and the decomposition by means of carbon dioxide usually requires considerable time. Methods have been devised, therefore, to decompose insoluble saccharates in other ways. One of the most common of such methods is the following:

*Decomposition of Saccharate by Means of Ammonium Nitrate.* — The saccharates of calcium are quickly decomposed by ammonium nitrate with formation of free sugar, calcium nitrate, and ammonia. The reaction for monocalcium saccharate is

$$\underset{\text{Saccharate}}{C_{11}H_{22}O_{11}CaO} + 2\,NH_4NO_3 + H_2O = \underset{\text{Sucrose}}{C_{11}H_{22}O_{11}} + Ca(NO_3)_2 + 2\,NH_4OH.$$

In carrying out the process 50 gms. of press cake are ground up with 15 gms. of ammonium nitrate and 100 c.c. of cold distilled water. The mixture is then washed into a 200-c.c. flask, clarified with a little lead-acetate solution, made up to volume, and polarized in the usual way.

An objection against the ammonium-nitrate method is the liberation of free ammonia, which in presence of the lead-clarifying agent may precipitate a part of the sucrose as lead saccharate. The free ammonia in some cases causes a darkening of the solution; contact with the brass fittings of polariscope tubes may also color the ammoniacal solution blue. Care should be exercised, therefore, to prevent contact of the solution with copper or brass during the analysis.

*Decomposition of Saccharate by Means of Zinc Nitrate.* — In order to eliminate the formation of free alkali Stanek* has proposed the employment of zinc nitrate for decomposing the saccharate. The reaction proceeds as follows:

$$\underset{\text{Monosaccharate}}{C_{11}H_{22}O_{11}CaO} + \underset{\text{Zinc nitrate}}{Zn(NO_3)_2} + H_2O = \underset{\text{Sucrose}}{C_{11}H_{22}O_{11}} + \underset{\text{Calcium nitrate}}{Ca(NO_3)_2} + \underset{\text{Zinc hydroxide.}}{Zn(OH)_2}$$

The precipitated zinc hydroxide is removed with the insoluble mineral matter of the cake and a perfectly neutral filtrate is obtained.

In carrying out the process a double normal weight (52 gms.) of press cake are thoroughly triturated with 100 c.c. of water; a few drops of phenolphthalein indicator are then added, and a neutral solution of zinc nitrate run in until the red color is just discharged. The volume is then completed to 210 c.c. (10 c.c. being allowed for the volume of insoluble cake and zinc hydroxide), and the solution filtered and polarized.

The methods, which have been described for polarizing products of the cane- and beet-sugar industry, may be applied equally well to the polarization of other sucrose-containing substances, such as maple and sorghum products, jellies, preserves, confections, etc. The same methods may also be applied to the polarization of substances which contain other sugars than sucrose, the only change necessary to make

* Z. Zuckerind. Böhmen, **34**, 161.

being in the constant for the normal weight. As an example of the application of saccharimetric methods to other sugars besides sucrose, the determination of milk sugar in milk is selected.

### Saccharimetric Determination of Lactose

**Polarization of Milk.*** — The normal weight of lactose for a saccharimeter with the Ventzke sugar scale may be taken as 32.9 gms. (see p. 197). Owing to the low percentage of lactose in milk (2 to 8 per cent) it is best to employ double the normal weight, and, as it is more convenient to measure the milk, tables have been prepared which give the volumes of milk corresponding to multiples of the normal weights for different saccharimeters. The following table gives the volumes of milk for 65.8 gms. which correspond to different specific gravities.

Table XLVI

*Giving the Volumes of Milk Corresponding to a Lactose Double Normal Weight*

| Specific gravity of milk. | Volume of milk for a lactose double normal weight (Ventzke scale). |
|---|---|
| | c.c. |
| 1.024 | 64.25 |
| 1.025 | 64.20 |
| 1.026 | 64.15 |
| 1.027 | 64.05 |
| 1.028 | 64.00 |
| 1.029 | 63.95 |
| 1.030 | 63.90 |
| 1.031 | 63.80 |
| 1.032 | 63.75 |
| 1.033 | 63.70 |
| 1.034 | 63.65 |
| 1.035 | 63.55 |
| 1.036 | 63.50 |

For ordinary purposes a pipette graduated to deliver 64 metric c.c. is sufficiently exact.

*Acid Nitrate of Mercury Solution.* — In clarifying milk for polarization acid nitrate of mercury is generally used. The reagent is prepared as follows: Dissolve metallic mercury in twice its weight of nitric acid of 1.42 sp. gr., and dilute with an equal volume of water.

*Mercuric-iodide Solution.* — Mercuric-iodide solution may also be used for clarification. The reagent is prepared by adding 33.2 gms. of potassium iodide to a solution of 13.5 gms. mercuric chloride in 20 c.c. of glacial acetic acid and 640 c.c. of water.

* Methods of Analysis A. O. A. C. Bull. 107 (revised), U. S. Bur. of Chem., p. 118.

In carrying out the process, the volume of milk corresponding to the lactose double normal weight is measured into a 102.6-c.c. flask. For clarification either 1 c.c. of the acid mercuric nitrate, or 30 c.c. of the mercuric-iodide solution may be used (an excess of either reagent does no harm). The liquid is shaken and then made up to a volume of 102.6 c.c., the extra 2.6 c.c. being the estimated volume of the precipitated casein, albumin, and fat. After mixing, the liquid is filtered and polarized in a 400-mm. tube; the scale reading divided by 4 gives the approximate percentage of lactose in the milk.

*Wiley and Ewell's* Double-dilution Method.* — The volume of precipitate in the preceding method varies according to the content of protein and fat so that the fixed estimate of 2.6 c.c. is not always accurate. For more exact purposes of analysis the double-dilution method of Wiley and Ewell may be used. The general principle of double dilution, due to Scheibler, has been considered on page 209.

Two separate double lactose-normal-weight portions of milk are introduced into a 100-c.c. and 200-c.c. flask respectively. The same volume of clarifying agent is then added to each flask and the volume completed to the mark. The solutions are shaken, filtered, and read in a 400-mm. tube. The reading of the 100-c.c. solution subtracted from 4 times the reading of the 200-c.c. solution gives the reading corrected for volume of precipitate, and this reading divided by 4 gives the percentage of lactose in the milk.

*Example.* — The saccharimeter readings (400-mm. tube) of a milk analyzed by the above method were 20.00 for the 100-c.c. flask and 9.80 for the 200-c.c. flask.

The reading corrected for volume of precipitate is then $(4 \times 9.80) - 20.00 = 19.20$, and the percentage of lactose is $19.20 \div 4 = 4.80$.

The volume of precipitate according to the above observations would be

$$\frac{100\,(20.0 - 19.2)}{20} = 4 \text{ c.c.}$$ (see p. 210).

*Leffman and Beam's Method.* — When the percentages of fat and protein are known in a milk, the volume of precipitate formed during clarification can be calculated according to Leffman and Beam † by the following method.

Calling the specific gravity of milk fat 0.93 the volume of precipitated fat is found by multiplying the grams of fat in the weight of sample by $\frac{1}{0.93} = 1.075$. In the same way the volume of the precipi-

* Analyst, **21**, 182. † "Analysis of Milk and Milk Products" (1896), p. 39.

tated protein-mercury compound is found by multiplying the grams of protein in the weight of sample by $\frac{1}{1.25} = 0.8$. The sum of the volumes of fat and protein is the volume in cubic centimeters of the precipitate.

For the polarization of evaporated or condensed milks the single lactose-normal-weight of substance is taken. The method of analysis in other respects is the same as described for ordinary milk.

The determination of lactose in milk by the saccharimeter is not considered upon the whole to be as accurate as by the gravimetric method of copper reduction. A considerable variation is frequently found in the determinations by the two methods. In ten comparative determinations of lactose in condensed milk by different collaborators of the Association of Official Agricultural Chemists* an average variation of $\pm$ 0.30 was found between the results by the optical and by the gravimetric method, the differences ranging from 0.03 to 0.90. In a series of comparative determinations by Patrick and Boyle† upon unsweetened condensed milks, the following results were obtained:

| Sample. | Lactose. | |
|---|---|---|
| | By polariscope, clarification with acid $Hg(NO_3)_2$. | By copper reduction, Soxhlet's method. |
| 1 | 10.07 | 10.04 |
| 2 | 10.19 | 10.51 |
| 3 | 10.57 | 10.69 |
| 4 | 9.97 | 10.15 |
| 5 | 8.71 | 9.20 |
| 6 | 9.00 | 9.37 |

The correction for volume of mercury precipitate in the above samples was made by the method of Leffman and Beam. It is seen that there is an average difference of about 0.25 between the two methods.

The cause of the occasional wide deviations between the results of the optical and gravimetric methods for determining lactose has been variously explained. The difference has been attributed by some to the presence of foreign optically active substances, such as unprecipitated proteids, organic acids, "animal gum," etc., but this has not been conclusively established. Differences due to variation in volume

* Proceedings A. O. A. C., 1906, 1907, Bulls. 105 and 116, U. S. Bur. of Chem.

† Bull. 105, U. S. Bur. of Chem., p. 109.

of precipitated fat and proteids are of course greater in case of condensed or evaporated milks.

**Polarization of Milk Sugar.** — The optical method for determining lactose is easily applied to the analysis of commercial milk-sugar, when other optically active compounds are absent. The lactose-normal-weight of sugar is made up to 100 c.c. with the addition of a little alumina cream; with dark-colored products containing milk sugar the solution of substance must be clarified, following the same methods and precautions as in the polarization of raw cane sugars. In polarizing milk sugar the saccharimeter reading must not be taken until mutarotation has disappeared; the solution of sugar is either allowed to remain in the tube until a constant reading is obtained or the mutarotation is destroyed by adding a few cubic centimeters of $N/10$ sodium carbonate solution at the time of making up to volume.

The methods of simple polarization described in the present chapter may obviously be applied to the polarization of products containing glucose, maltose, and other sugars. But in practical work it is found that such sugars generally occur in mixtures with other carbohydrates, and the methods for their determination are accordingly given elsewhere.

### Influence of Temperature upon Saccharimetric Observations*

Before concluding this chapter upon methods of simple polarization, the influence of changes in temperature upon the accuracy of saccharimetric observations should be considered.

It has been shown (p. 127) that with an increase in temperature the specific rotation of sucrose undergoes a decrease and the rotatory power of the quartz compensation an increase, the combined effect of all influences producing a decrease in the saccharimeter reading of a normal weight of pure sucrose of 0.03° V. for 1° C. increase in temperature, and that for temperatures between 20° and 30° C. the general equation $V^{20^\circ} = V^t\{1 + 0.0003\,(t - 20)\}$ may be used for changing the Ventzke reading ($V^t$) of pure sucrose at any temperature $t$ to the reading at 20°.

**Saccharimeter Temperature Corrections.** — The employment of a temperature correction, similar to the above, was made by the

* For a full discussion of this question with bibliographic references see paper by Browne, "The Use of Temperature Corrections in the Polarization of Raw Sugars and Other Products upon Quartz Wedge Saccharimeters," read before Section V, Seventh International Congress of Applied Chem., London, 1909, also in J. Ind. and Eng. Chem. I, 567, and Z. Ver. Deut. Zuckerind., **59**, 404.

United States Treasury Department in 1897, in its polarization of sugars assessed for duty. The right of the Treasury Department to make such corrections in the observed saccharimeter readings was contested in the courts by several importers of sugar, who founded their case largely upon the claim that the rotation of pure sucrose is not appreciably affected by changes in temperature. The chemists representing the government were successful, however, in showing that the specific rotation of sucrose is thus affected, and after a final appeal to the United States Supreme Court the case of the importers was dismissed for want of jurisdiction.*

The decision of the courts, which apparently justified the use of temperature corrections established for pure sucrose in correcting the polarization of all grades of raw sugars, has unfortunately seemed to many chemists sufficient authorization to use such corrections indiscriminately in the polarization of any and every kind of sugar-containing material. Since the saccharimetric reading of a raw sugar or other impure product is simply an expression of the sum of the optical activities of the various constituents, sucrose, glucose, fructose, organic acids, gums, etc., it is evident that a system of temperature corrections which shall give the saccharimeter reading that would be obtained at 20° C., must correct for the variations produced by temperature in the specific rotation of all the optically active ingredients and not of the sucrose alone.

*Wiley's Temperature Correction Table.* — Wiley† has prepared a temperature table for correcting the readings of quartz wedge saccharimeters which is based upon the variations in the Ventzke scale reading of normal and fractional normal weights of pure sucrose. This table has a range from 75° V. to 100° V. for temperatures between 4° C. and 40° C.; the corrections are to be subtracted from the observed readings, when the temperature of polarization is below and to be added when the temperature is above that of standardization.

*United States Treasury Department Method of Temperature Corrections.* — The method of temperature corrections devised by the Office of Weights and Measures of the United States Coast and Geodetic Survey and adopted by the United States Treasury Department for use in the Custom-House laboratories, consists in increasing or diminishing the saccharimeter reading of each sugar solution by the variation

* For testimony in this case see "Transcript of Record," U. S. Supreme Court, the American Sugar Refining Company, *vs.* The United States.

† J. Am. Chem. Soc., **21**, 568.

in reading which a standard quartz plate shows from the computed sugar value of this plate for the temperature of observation.

The following report gives the temperature corrections in sugar degrees for a quartz control plate tested by the United States Bureau Standards.

DEPARTMENT OF COMMERCE AND LABOR, BUREAU OF STANDARDS, WASHINGTON

ACCOMPANYING REPORT OF TEMPERATURE CORRECTIONS IN SUGAR DEGREES FOR QUARTZ CONTROL PLATE 233–B.S. 1910.

| Degrees centigrade. | Sugar value. | Degrees centigrade. | Sugar value. | Degrees centigrade. | Sugar value. | Degrees centigrade. | Sugar value. |
|---|---|---|---|---|---|---|---|
| 13.0° | 90.04° | 20.0° | 90.25° | 25.0° | 90.40° | 30.0° | 90.55° |
| 14.0 | 90.07 | 20.5 | 90.27 | 25.5 | 90.42 | 30.5 | 90.57 |
| 15.0 | 90.10 | 21.0 | 90.28 | 26.0 | 90.43 | 31.0 | 90.58 |
| 16.0 | 90.13 | 21.5 | 90.30 | 26.5 | 90.45 | 31.5 | 90.60 |
| 17.0 | 90.16 | 22.0 | 90.31 | 27.0 | 90.46 | 32.0 | 90.61 |
| 17.5 | 90.18 | 22.5 | 90.33 | 27.5 | 90.48 | 32.5 | 90.63 |
| 18.0 | 90.19 | 23.0 | 90.34 | 28.0 | 90.49 | 33.0 | 90.64 |
| 18.5 | 90.21 | 23.5 | 90.36 | 28.5 | 90.51 | 34.0 | 90.67 |
| 19.0 | 90.22 | 24.0 | 90.37 | 29.0 | 90.52 | 35.0 | 90.70 |
| 19.5 | 90.24 | 24.5 | 90.39 | 29.5 | 90.54 | 36.0 | 90.73 |

If the polarization temperature is above 20°C., add to the reading the difference between the reading of the plate and the sugar value of the plate at the polarization temperature shown by the above table. If the polarization temperature is below 20°C., subtract the correction.

It will be noted from this table that the variation of 0.030° V. per 1° C., for the reading of a normal weight of pure sucrose, is applied without change to a plate testing 90.25° V. at 20° C. The true temperature correction for a sucrose solution reading 90.25° V. upon the saccharimeter would of course be $0.030 \times 0.9025 = 0.027$ per 1° C. The correction table is strictly true therefore only for sugar solutions polarizing 100° V. at 20° C. It would be wrong in principle to apply such corrections to sucrose solutions testing 80° V. or 50° V. or 20° V. since in the latter instances the corrections are only 80 per cent, 50 per cent, and 20 per cent, respectively, of the correction for a 100° V. sucrose solution. The correction formula $V^{20°} = V^{t} \{1 + 0.0003\ (t - 20)\}$ or the equivalent corrections of Wiley's table are, therefore, to be preferred to the method used by the United States Treasury Department, when it is desired to correct the polarizations of pure sucrose solutions for change in temperature.

*Errors Involved in Use of Saccharimeter Temperature Corrections.* — The probable errors involved in the use of the above methods for cor-

recting polarizations may be seen from the following diagram (Fig. 144), which gives the correction for pure sucrose solutions, and the approximate corrections for solutions of sugar-beet and sugar-cane products (according to results obtained by Browne *), to be applied to the readings of the Ventzke scale for 1° C. increase in temperature.

It will be seen that the correction for beet products is much nearer

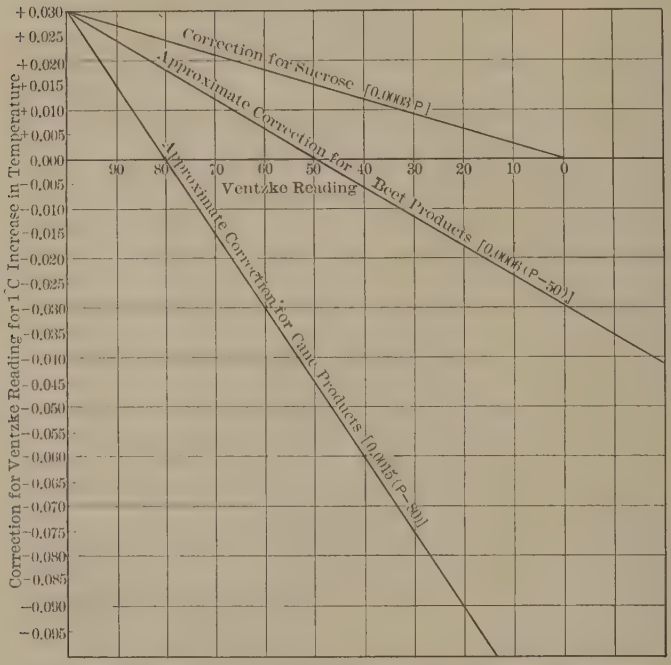



Fig. 144. — Diagram for correcting polarizations of sugar products for changes in temperature.

the correction for pure sucrose than that for cane products. This is due to the fact that raw cane products contain a larger amount of fructose, the change in specific rotation of which towards the right, as the temperature increases, compensates to a greater or less degree the change in specific rotation of sucrose towards the left. This is made more evident in Table XLVII, which gives the polarization and composition of various grades of raw cane sugar.

* J. Ind. Eng. Chem., **1**, 567.

TABLE XLVII

*Showing Effect of Increase in Temperature upon the Polarization of Sugar-cane Products, Browne* †

| No. | Description of sugar. | Polarization. | Sucrose | Invert sugar. | Water. | Ash. | Organic non-sugar by difference. | Change in polarization for 1° C. increase. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Found. | By formula $0.0003\ P$. |
| | | | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | | |
| 1 | Java | 98.55 | 98.74 | 0.64 | 0.19 | 0.21 | 0.22 | −0.0311 | −0.0296 |
| 2 | Peru | 97.45 | 97.61 | 0.52 | 0.45 | 0.46 | 0.96 | −0.0301 | −0.0292 |
| 3 | Cuba | 97.15 | 97.38 | 0.78 | 1.03 | 0.31 | 0.50 | −0.0276 | −0.0291 |
| 4 | San Domingo | 96.15 | 96.61 | 1.53 | 0.85 | 0.48 | 0.53 | −0.0230 | −0.0288 |
| 5 | Cuba | 94.50 | 95.05 | 1.83 | 1.97 | 0.67 | 0.48 | −0.0212 | −0.0287 |
| 6 | Cuba | 93.75 | 94.44 | 2.29 | 1.83 | 0.55 | 0.89 | −0.0160 | −0.0281 |
| 7 | Philippine | 89.20 | 90.59 | 4.63 | 2.11 | 1.27 | 1.40 | −0.0110 | −0.0268 |
| 8 | Louisiana | 87.60 | 89.00 | 4.67 | 2.30 | 3.17 | 0.86 | −0.0106 | −0.0263 |
| 9 | Philippine | 82.40 | 84.64 | 7.45 | 3.49 | 1.85 | 2.57 | 0.0000 | −0.0247 |
| 10 | Louisiana | 79.65 | 81.69 | 6.80 | 4.84 | 4.21 | 2.46 | +0.0068 | −0.0239 |
| 11 | Cuba | 67.70 | 71.05 | 11.18 | 6.70 | 3.75 | 7.32 | +0.0286 | −0.0203 |
| | Louisiana molasses* | 20.06 | 29.58 | 30.09 | 23.62 | 8.24 | 8.47 | +0.1120 | −0.0060 |
| | Calculated mixtures of sucrose and cane molasses. | | | | | | | | |
| | Sucrose, per cent. / Molasses, per cent. | | | | | | | | |
| | 95 / 5 | 96.00 | 96.50 | 1.50 | 1.10 | 0.40 | 0.50 | −0.0229 | −0.0288 |
| | 90 / 10 | 92.00 | 93.00 | 3.00 | 2.20 | 0.80 | 1.00 | −0.0158 | −0.0276 |
| | 85 / 15 | 88.00 | 89.50 | 4.50 | 3.30 | 1.20 | 1.50 | −0.0087 | −0.0264 |
| | 80 / 20 | 84.00 | 86.00 | 6.00 | 4.40 | 1.60 | 2.00 | −0.0016 | −0.0252 |
| | 75 / 25 | 80.00 | 82.50 | 7.50 | 5.50 | 2.00 | 2.50 | +0.0055 | −0.0240 |
| | 70 / 30 | 76.00 | 79.00 | 9.00 | 6.60 | 2.40 | 3.00 | +0.0126 | −0.0228 |

* Average of 4 samples.

Raw sugars can be regarded as simple mixtures of sucrose crystals and molasses, and the results in the second part of the table calculated for various theoretical mixtures of sucrose and exhausted cane molasses agree closely with those observed for the different raw sugars.

The observations by Browne in Table XLVII have also been confirmed by Wiley and Bryan ‡ who obtained very similar figures upon different grades of raw cane sugar.

The effect of temperature upon the polarization of American beet sugar and molasses is shown in Table XLVIII.

† J. Ind. Eng. Chem., **1**, 567.

‡ Z. Ver. Deut. Zuckerind., **59**, 916.

TABLE XLVIII

*Showing Effect of Increase in Temperature upon the Polarization of Sugar-beet Products, Browne* †

| No. | Product. | Polarization. | Sucrose. | Raffinose. | Invert sugar. | Water. | Ash. | Organic non-sugar by difference. | Change in polarization for 1° C. increase. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Found. | Formula $0.0003\ P$. |
| | | | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | | |
| 1 | Beet sugar.. | 91.25 | ..... | ..... | ..... | ..... | ..... | ..... | −0.0276 | −0.0274 |
| 2 | Beet sugar.. | 86.60 | ..... | ..... | ..... | ..... | ..... | ..... | −0.0263 | −0.0260 |
| 3 | Beet sugar.. | 85.50 | ..... | ..... | ..... | ..... | ..... | ..... | −0.0214 | −0.0257 |
| 4 | Beet molasses* | 51.22 | 48.13 | 1.72 | 0.94 | 19.86 | 7.62 | 21.74 | −0.0053 | −0.0154 |

Calculated mixtures of sucrose and beet molasses.

| Sucrose, per cent | Molasses, per cent. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 90 | 10 | 95.00 | 94.80 | 0.15 | 0.10 | 2.0 | 0.75 | 2.20 | −0.0275 | −0.0285 |
| 80 | 20 | 90.00 | 89.60 | 0.30 | 0.20 | 4.0 | 1.50 | 4.40 | −0.0250 | −0.0270 |
| 70 | 30 | 85.00 | 84.40 | 0.45 | 0.30 | 6.0 | 2.25 | 6.60 | −0.0225 | −0.0255 |
| 60 | 40 | 80.00 | 79.20 | 0.60 | 0.40 | 8.0 | 3.00 | 8.80 | −0.0200 | −0.0240 |

* Average of 3 samples.

It will be seen from the above that the temperature formula $P^{20} = P^t [1 + 0.0003\ (t - 20)]$, or the corresponding corrections of the Wiley table, can be applied without serious error to practically all grades of beet sugar and to those grades of cane sugar polarizing over 96. As the polarization of raw cane sugars falls below 96, and the percentage of invert sugar (or fructose) increases, the effect of change in temperature upon the rotation of the latter begins to lower appreciably the temperature coefficient for the rotation of sucrose until, at a point about 80° V., the two influences — that of the temperature upon the fructose and other impurities and that of the temperature upon the sucrose and quartz wedges of the instrument — exactly counterbalance one another.‡ Under these conditions a sugar will polarize the same at all temperatures. Below 80° V. the temperature coefficient for the rotation of the sucrose in raw cane sugars is usually more than

† J. Ind. Eng. Chem., **1**, 567.

‡ The calculation upon page 128 shows that the proportion of fructose to sucrose for equilibrium between their temperature coefficients is 3.13 to 100.0.

counterbalanced, the result being that the polarization of these sugars *increases* with elevation of temperature. This increase continues, as the polarization diminishes (the percentage of fructose and other impurities being greater), until, at a polarization of about + 20 for exhausted cane molasses, an increase of 1° C. in temperature causes an increase of over 0.1° V. in the saccharimeter reading.

*Correction of Polarizations for the Combined Influence of Temperature upon the Rotation of Sucrose and Invert Sugar.* — Since the ingredient of sugar products, whose polarization is most susceptible to the influence of temperature, is invert sugar, a more accurate method of correcting saccharimeter readings is to combine the temperature coefficients of sucrose and invert sugar as by the formula: $P^{20} = P^t + 0.0003\,S\,(t - 20) - 0.0045\,I\,(t - 20)$ in which $P^t$ is the polarization at $t°$ C., $S$ the percentage of sucrose and $I$ the percentage of invert sugar.

If the percentage of invert sugar is unknown the temperature correction for converting polarizations to 20° C. may be determined approximately by the following empirical equations:

For cane products, $P^{20} = P^t + 0.0015\,(P^t - 80)\,(t - 20)$,
For beet products, $P^{20} = P^t + 0.0006\,(P^t - 50)\,(t - 20)$.

Such formulæ as the above while more accurate than corrections which are based upon the temperature coefficients of pure sucrose, fail to give accurate results upon many individual products whose composition differs from that of the average type.

**Polarization at Constant Temperature.** — It is evident from the foregoing that the method of applying temperature corrections established for pure sucrose to the polarization of sugar products in general is faulty. Since it is impossible to devise a simple reliable method of temperature corrections that can be applied to the polarization of all kinds of substances, the one means of securing uniformity and accuracy in saccharimetric work is to make all polarizations at the temperature at which the instruments are standardized. Custom-house laboratories, arbitration laboratories, and all other laboratories, upon the results of which great interests are involved, should be equipped with cooling and warming apparatus for maintaining a uniform standard temperature throughout the year.

The New York Sugar Trade Laboratory was the first testing laboratory in the United States to follow out the requirements of the International Commission for Uniform Methods of Sugar Analysis and make all polarizations at 20° C. The laboratory room and polarizing cabinet used for this purpose are insulated. In warm weather the air

is circulated by an electric fan through ducts over cooling coils, fresh air being introduced from outside according to the needs of ventilation. A small ammonia compressor driven by an electric motor serves for the work of refrigeration. The temperature can be controlled either

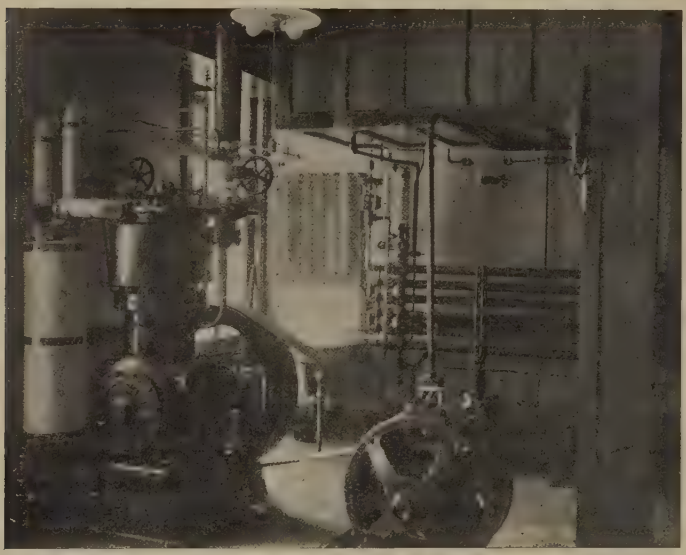


Fig. 145. — Refrigerating machine for constant temperature polarization (New York Sugar Trade Laboratory).

automatically by means of a thermostat which operates dampers regulating the passage of air to and from the cooling box, or directly by means of the rheostats controlling the speed of compressor and ventilating fan. The general arrangement of the equipment is shown in Figs. 145 and 121.

# CHAPTER X

## METHODS OF INVERT OR DOUBLE POLARIZATION

The methods of direct polarization, as previously explained, give percentage of sucrose only in the absence of other optically active substances. To determine the percentage of sucrose when other optically active substances are present, the method of inversion or double polarization is used, the principle of which may be understood from the following.

**Law of Inversion.** — When a solution of sucrose is acted upon by some inverting agent, such as an acid or the enzyme invertase, the sucrose molecule is broken up or inverted, giving rise, by the addition of one molecule of water, to one molecule each of glucose and fructose, the mixture of these two sugars in equal amounts being termed invert sugar. This reaction, known as hydrolysis or inversion, is expressed by the following equation:

$$\underset{\text{Sucrose (342)}}{C_{12}H_{22}O_{11}} + \underset{\text{Water (18)}}{H_2O} = \underbrace{\underset{\text{Glucose (180)}}{C_6H_{12}O_6} + \underset{\text{Fructose (180)}}{C_6H_{12}O_6}}_{\text{Invert Sugar (360)}}.$$

It is seen from the above that one part of sucrose is converted into $\frac{360}{342} = 1.05263$ parts of invert sugar. Calling the specific rotation at 20° C. + 66.5 for sucrose, and − 20.00 for invert sugar (p. 174), the relation of the optical activity of one part sucrose before and after inversion will be $+ 66.5 : 1.05263 (- 20.00) = 66.5 : - 21.0526$ or a decrease of 87.5526 in specific rotation. This decrease for one degree of the saccharimeter scale would therefore be $\frac{87.5526}{66.5} = 1.3166$. The general law of inversion* as applied to the determination of sucrose may then be stated as follows:

The total decrease in the saccharimeter reading at 20° C. of the normal weight of product after inversion divided by 1.3166 gives the percentage of sucrose when no other optically active ingredient is hydrolyzed and when the inverting agent produces no change in the specific rotation of the other optically active constituents present.

* For a fuller discussion of the laws of inversion see page 659.

The enzyme invertase fulfills most perfectly the conditions above named, and when this is used as the inverting agent the percentage of sucrose in mixtures with glucose, fructose, invert sugar, maltose, milk sugar, etc., may be determined very closely by use of the factor 1.3166. The inverting agent most commonly used in optical analysis is not invertase, however, but hydrochloric acid, the presence of which, as shown on page 185, has a most pronounced influence in increasing the specific rotation of fructose. When hydrochloric acid is used for inverting, the factor 1.3166 must be modified according to the amount of acid used for inverting, the concentration of the sugar solution, and the manner of conducting the inversion. The extreme susceptibility of fructose to changes in specific rotation and composition makes it necessary in employing any method of inversion to adhere most rigidly to the rules of procedure prescribed.

### The Clerget Method of Inversion

The method of inversion for determining sucrose was devised in 1849, by Clerget,* who found that a solution of the French normal weight of pure sucrose in 100 c.c., reading + 100 degrees upon the saccharimeter, gave after inversion with hydrochloric acid a reading of − 44 degrees at 0° C. or − 34 degrees at 20° C. The total difference between the readings before and after inversion, correcting for the influence of temperature, is expressed by the quantity

$$100 - (-44) - \frac{t}{2} = 144 - \frac{t}{2},$$

$t$ being the temperature of the inverted solution at polarization.

If $D$ represents the algebraic difference $(P - P')$ between the direct polarization $(P)$ and the invert polarization $(P')$ of a given product, then the percentage $(S)$ of sucrose by Clerget's formula is expressed by the equation $S = \frac{100\,D}{144 - \frac{t}{2}}$. If the invert polarization is made at 20° C. the equation becomes $S = \frac{100\,D}{134}$ or $\frac{D}{1.34}$. The factor 1.34 is considerably greater than the factor 1.3166 for pure aqueous solutions of invert sugar.

Tuchschmid† who subjected the Clerget process to an exhaustive analysis, arrived at the following formula,

$$S = \frac{100\,D}{144.16035 - 0.50578\,t}.$$

* Compt. rend., **16,** 1000; **22,** 1138; **23,** 256; **26,** 240; Ann. chim. phys. [3], **26,** 175.

† Z. Ver. Deut. Zuckerind., **20,** 649.

The original Clerget formula does not differ sufficiently from this to warrant the greater labor of calculation involved in the use of the long decimals.

If the direct and invert readings are made upon a polarimeter with circular degrees the Clerget formula would be, for the German normal weight (1° sugar scale = 0.34657 circular degrees),

$$\frac{100\,D}{.34657\,(144 - .5\,t)} = \frac{100\,D}{49.906 - 0.173\,t};$$

for the French normal weight (1° sugar scale = 0.21719 circular degrees),

$$\frac{100\,D}{.21719\,(144 - .5\,t)} = \frac{100\,D}{31.275 - 0.109\,t}.$$

One gram of sucrose dissolved to 100 metric cubic centimeters gives a direct reading of $\frac{34.657}{26} = 1.333$ circular degrees and an invert reading of $-\frac{15.249}{26} = -0.5865$ circular degrees at 0° C; the grams of sucrose ($C$) in 100 c.c. of any solution may be found from the polarimeter reading before and after inversion by the equation

$$C = \frac{P - P'}{\frac{(49.906 - 0.173\,t)}{26}} = \frac{P - P'}{1.9195 - 0.0067\,t}.$$

The Clerget formulæ, given above, are to be employed only when the following method of inversion prescribed by Clerget is followed. After taking the direct polarization (p. 202), the clarified solution remaining is filled up to the 50-c.c. graduation mark of a flask graduated at 50 and 55 c.c.; concentrated hydrochloric acid is then added to the 55-c.c. mark, a thermometer is inserted, and the flask slowly warmed until the temperature reaches 68° C., 15 minutes being taken in the heating.* The solution is then quickly cooled, filtered if necessary, and polarized as nearly as possible at the original temperature of making up to volume. The polariscope reading for a 200-mm. tube of solution must be increased by $\frac{1}{10}$ to correct for the dilution with acid. The reading of the inverted solution is sometimes made in a 220-mm. tube, when no correction for dilution is needed.

* The addition of the acid causes an elevation of 2° to 3° C. in temperature; there is also a slight loss from evaporation during the inversion. It is, therefore, better to control the temperature by inserting the thermometer in a 50–55 c.c. flask filled with water and placed in the bath with the solutions undergoing inversion. After cooling to room temperature, the volumes are readjusted to 55 c.c.

In carrying out the inversion special attention must be paid to all details. If the temperature of 68° C., or the time of 15 minutes, is exceeded, a partial destruction of fructose may result; if the temperature of 68° C. is not reached, or if the time of heating is less than 15 minutes, some of the sucrose may escape inversion. Care must also be taken to maintain a constant temperature in the polarization tube during the reading. Even a slight warming of the tube, as from handling, will affect the observation. A polarization tube provided with a jacket for circulation of water at the desired temperature is very desirable for polarizing inverted solutions. (See Fig. 111.)

**Herzfeld's Modification of the Clerget Method.** — The original method of Clerget has been variously modified from time to time in order to diminish the danger of destroying fructose and to secure better uniformity of conditions. The inversion method of Herzfeld,* which is the one most generally employed at present, is as follows:

The half normal weight (13.00 gms.) of product is transferred with 75 c.c. of water into a 100-c.c. flask; after solution of soluble matter, 5 c.c. of hydrochloric acid of sp. gr. 1.188 are added, a thermometer is introduced and the flask placed in a water bath heated to between 72° and 73° C. As soon as the thermometer in the flask indicates 69° C. (2.5 to 5 minutes) the solution is kept at this temperature for exactly 5 minutes, rotating the flask gently at frequent intervals to secure even distribution of the heat. The entire time of heating, according to the length of the preliminary period, will vary thus from $7\frac{1}{2}$ to 10 minutes, and should never exceed 10 minutes. When the 5-minute heating at 69° C. is completed the flask is cooled as quickly as possible to 20° C., the thermometer is rinsed from adhering sugar solution and the volume made to 100 c.c. After mixing and filtering, the solution is polarized with all the precautions previously mentioned. The polariscope reading is doubled to obtain the correct invert reading for a normal weight of substance.

The invert reading for 26 gms. of chemically pure sucrose under the above conditions is −42.66° V. at 0°, or −32.66° V. at 20° C. The Clerget formula, according to Herzfeld's modification, is then expressed by the equation

$$S = \frac{100\,D}{142.66 - 0.5\,t};$$

or, if the polarization be made always at 20° C., by

$$S = \frac{100\,D}{132.66} = 0.7538\,D.$$

* Z. Ver. Deut. Zuckerind. (1888), **38**, 699.

*Effect of Concentration on the Clerget Factor.* — The factor 132.66 in the preceding equation is correct only for a solution containing the half normal weight of sugar to 100 c.c. For other concentrations than this the value of the invert reading will vary according to the general formula $P'^{20°} = -(31.78 + 0.0676\,c)$, or $P'^{0°} = -(41.78 + 0.0676\,c)$, in which $P'$ is the invert reading upon the Ventzke scale and $c$ the grams of sucrose in 100 c.c. The following table gives the value of the factor 142.66 in the equation $S = \frac{100\,D}{142.66 - 0.5\,t}$ for different concentrations of sucrose.

TABLE XLIX

*Giving Clerget Factors at Different Concentrations of Sucrose for Herzfeld's modified Method*

| Grams sucrose in 100 c.c. | Factor. | Grams sucrose in 100 c.c. | Factor. |
|---|---|---|---|
| 1 | 141.85 | 14 | 142.73 |
| 2 | 141.91 | 15 | 142.79 |
| 3 | 141.98 | 16 | 142.86 |
| 4 | 142.05 | 17 | 142.93 |
| 5 | 142.12 | 18 | 143.00 |
| 6 | 142.18 | 19 | 143.07 |
| 7 | 142.25 | 20 | 143.13 |
| 8 | 142.32 | 21 | 143.20 |
| 9 | 142.39 | 22 | 143.27 |
| 10 | 142.46 | 23 | 143.33 |
| 11 | 142.52 | 24 | 143.40 |
| 12 | 142.59 | 25 | 143.47 |
| 13 | 142.66 | 26 | 143.54 |

Instead of the above correction table the following general formula has been proposed by Herzfeld:*

$$S = \frac{100\,(P - P')}{141.84 + 0.05\,N - 0.5\,t},$$

in which $P$ and $P'$ are the direct and invert polarizations for a normal weight of substance and $N$ the scale reading of the inverted solution. This formula assumes that the value $N$ always bears a constant ratio to the concentration of sucrose, which is of course only true when other optically active substances are absent.

*Example.* — The application of the above Herzfeld formula is best illustrated by an example: 26.00 gms. of a sugar sirup dissolved to 100 true c.c. at 20° C. gave a direct reading in a 200-mm. tube of + 60.00 ($P$). 13.00 gms. of

* Z. Ver. Deut. Zuckerind., **40**, 194.

this same sirup inverted according to Herzfeld's method gave a reading of 9.7 ($N$) at 20° ($t$) upon the negative scale, $-9.7 \times 2 = -19.4$ ($P'$). Substituting these values in the formula, we obtain

$$S = \frac{100\,[+60 - (-19.4)]}{141.84 + (0.05 \times 9.7) - (0.5 \times 20)} = 60 \text{ per cent},$$

the amount of sucrose present in the sirup.

If the direct and invert polarizations be made at 20° C. the Clerget and Herzfeld formulæ become simplified as follows:

$$\text{Clerget formula} = \frac{100\,(P - P')}{144 - \frac{2}{2}\,0} = 0.7463\,(P - P');$$

$$\text{Herzfeld formula} = \frac{100\,(P - P')}{142.66 - \frac{2}{2}\,0} = 0.7538\,(P - P').$$

The values of the Herzfeld factor in the simplified formula for temperatures between 10° and 40° C. are given in Table L.

TABLE L

*Giving the Inversion Factors for Herzfeld's Modification of Clerget's Method at Different Temperatures*

| Temperature. | Factor. | Temperature. | Factor. | Temperature. | Factor. |
|---|---|---|---|---|---|
| 10° C. | 0.7264 | 20° C. | 0.7538 | 30° C. | 0.7833 |
| 11 | 0.7290 | 21 | 0.7566 | 31 | 0.7864 |
| 12 | 0.7317 | 22 | 0.7595 | 32 | 0.7895 |
| 13 | 0.7344 | 23 | 0.7624 | 33 | 0.7926 |
| 14 | 0.7371 | 24 | 0.7653 | 34 | 0.7957 |
| 15 | 0.7398 | 25 | 0.7682 | 35 | 0.7989 |
| 16 | 0.7426 | 26 | 0.7712 | 36 | 0.8021 |
| 17 | 0.7454 | 27 | 0.7742 | 37 | 0.8053 |
| 18 | 0.7482 | 28 | 0.7772 | 38 | 0.8086 |
| 19 | 0.7510 | 29 | 0.7802 | 39 | 0.8119 |
| ..... | ..... | ..... | ..... | 40 | 0.8152 |

The inversion method of Herzfeld gives correct results only when the prescribed conditions of concentration, amount of acid, volume, temperature and time of inversion are carefully followed. The temperature of inversion for the 5-minute period should be maintained at exactly 69° C. if possible; a variation of 1° C. from this temperature is found to produce a difference of over 0.1 in the calculated percentage of sucrose. The extreme sensibility of fructose to decomposition during inversion and its wide fluctuation in optical rotation with slight changes of temperature necessitate the greatest care in manipulation. Neglect of this precaution is a frequent cause of variation between the results of different analysts.

*Inversion at Ordinary Temperature.* — The dangers of too high or too prolonged heating in the Clerget determination may be avoided by inverting at the ordinary laboratory temperature. The time necessary to invert a half-normal weight (13 gms.) of sucrose in 100 c.c. of solution employing hydrochloric acid of 1.18 sp. gr. was found by Hammerschmidt * to be as follows:

| Temperature. | 5 c.c. HCl. | 10 c.c. HCl. |
|---|---|---|
| °C. | Hours. | Hours. |
| 10 | 225 | 94 |
| 15 | 101 | 44 |
| 20 | 47 | 20 |
| 25 | 23 | 10 |
| 30 | 11.6 | 5 |

The method of Tolman† for cold acid inversion is to place 50 c.c. of a solution containing the half-normal, or normal, weight of substance in a 100-c.c. graduated flask, add 5 c.c. of strong hydrochloric acid, allow to stand at room temperature (above 20° C.) for 20 to 24 hours, make up to 100 c.c. and polarize. At 25° C. the inversion is complete in about 10 hours and at 20° C. in about 20 hours. The Clerget factor for a half-normal weight (13 gms.) of sucrose inverted in the cold was found by Tolman to be 142.88.

*Effect of Amount of Acid on the Clerget Factor.* — The effect of varying the quantity of hydrochloric acid used for inversion upon the Clerget factor was studied by Hammerschmidt,* who obtained the following invert readings at 20° C. for a normal weight of pure sucrose, using 5 c.c., 10 c.c., 15 c.c., and 20 c.c. of hydrochloric acid per 100 c.c.

| | 5 c.c. | 10 c.c. | 15 c.c. | 20 c.c. |
|---|---|---|---|---|
| Reading of normal weight, (Degrees Ventzke) | −34.00 | −35.04 | −35.95 | −36.80 |
| Reading of ½ normal weight × 2, (Degrees Ventzke) | −33.00 | −34.12 | −35.15 | −36.03 |

It will be noted that there is a pronounced but diminishing increase in the invert reading with the addition of each 5 c.c. of acid.

Results similar to those of Hammerschmidt were obtained by Tolman,† who found for a solution of invert sugar made up to volume with no hydrochloric acid a reading of −23.0° V., for the same amount of invert sugar solution made up with 5 c.c. hydrochloric acid a reading of −24.2, and for a third similar portion made up with 10 c.c. hydrochloric acid a reading of −25.0.

* Z. Ver. Deut. Zuckerind, **40,** 465. † Bull. 73, U. S. Bur. Chem., p. 69.

*Effect of Fructose on the Clerget Factor.* — Owing to the influence of hydrochloric acid upon the polarization of fructose a Clerget formula based upon the inversion of pure sucrose by means of this acid is not absolutely correct when applied to the analysis of impure products containing invert sugar, since the specific rotation of fructose is different in the neutral and acid solutions before and after inversion. A considerable error is introduced, in fact, if the Clerget formula established for pure sucrose be employed in the examination of molasses, honey, jam, jelly, and other materials containing considerable fructose.

*Effect of Amino Compounds on the Clerget Factor.* — The hydrochloric acid used for inversion may also affect the polarization of other ingredients than fructose. Low-grade molasses, plant extracts, and other sugar-containing materials frequently contain considerable quantities of optically active amino compounds such as asparagine, aspartic acid, glutaminic acid, leucine, isoleucine, etc., the optical activity of which varies with the alkalinity and acidity of the solution. This may be seen from the following table which gives the approximate specific rotations of several amino derivatives in alkaline solution, in water, and in hydrochloric acid.

TABLE LI.

*Approximate Value for* $[\alpha]_D$.

| | In presence of NaOH. | In water. | In presence of HCl. |
|---|---|---|---|
| Asparagine | − 8 | − 6 | +34 |
| Aspartic acid | − 9 | + 4 | +34 |
| Glutaminic acid | −68 | +10 | +20 |
| Leucine | + 7 | ..... | +17 |
| Isoleucine | + 11 | +10 | +37 |

The influence of such variations upon the Clerget calculation is illustrated in the work of Andrlik and Stanek * who showed that a 1 per cent solution of glutaminic acid gave a reading of −1.45° V. in presence of lead subacetate, −0.35° V. in water alone, and +1.77° V. in dilute hydrochloric acid. In the case of an osmose water from a beet-sugar factory the direct polarization was 14.75° V. in alkaline, 14.85° V. in neutral, and 15.80° V. in acid solution. Ehrlich† had previously also called attention to the large errors in the Clerget method due to the presence of amino compounds.

* Z. Zuckerind. Böhmen, **31,** 417.

† Z. Ver. Deut. Zuckerind., **53,** 809.

**Clerget Modifications for Impure Sugar Products.** — It is evident that to overcome the variations in specific rotation of fructose, amino compounds, etc., which occur in the presence and absence of hydrochloric acid, the original method of Clerget must be considerably modified in the case of impure products. Several such modifications of the method have in fact been devised and these for convenience may be grouped into two general classes. I. Clerget modifications which attempt to equalize the conditions before and after inversion with hydrochloric acid. II. Clerget modifications which employ an inverting agent free from the objections of hydrochloric acid.

Among the modifications of Class I may be mentioned the following.

(1) *Neutralizing the Free Acid after Inversion before Making the Invert Polarization.* — This modification is best carried out in the Herzfeld process of inversion. After cooling the solution the free hydrochloric acid is carefully neutralized by means of sodium hydroxide, using phenolphthalein as indicator, and avoiding any excess of alkali. After neutralizing, the volume is completed to 100 c.c. at 20° C. and the invert polarization made in the usual way. When this modification is used sucrose is calculated by the formula

$$S = \frac{100(P - P')}{141.7 - 0.5\,t}.$$

While the method of neutralization removes the influence of the free hydrochloric acid, a new disturbing factor is introduced by the formation of a considerable amount of sodium chloride. The latter, however, is of minor importance compared with the free acid.

(2) *Making the Direct Polarization in Presence of Hydrochloric Acid and Urea.* — This modification, due to Andrlik and Stanek,* is based upon the retarding influence which urea (or betaine) exercises upon the inversion of sucrose with hydrochloric acid in the cold. Fifty cubic centimeters of the solution for the direct polarization are made up to 100 c.c. with a solution containing 5 gms. urea and 5 c.c. strong hydrochloric acid per 50 c.c. of reagent. After mixing, the solution is filtered and polarized as quickly as possible. It is claimed by the authors of the method that a sufficient interval (7 to 10 minutes) elapses before inversion is noticeable to make the direct polarization. While this claim may be true for certain classes of products, it is certainly not the case with substances rich in sucrose. The following experiment shows a comparison of the rate of inversion of 13 gms. of sucrose at 20° C. in presence of 5 c.c. strong hydrochloric acid and in presence of 5 c.c. strong hydrochloric acid plus 5 gms. urea in 100 c.c. of solution.

* Z. Zuckerind. Böhmen, **31**, 417.

TABLE LII

*Showing Influence of Urea upon the Rate of Inversion of Sucrose*

| Time. | Inversion with 5 c.c. HCl. | | Inversion with 5 c.c. HCl + 5 gms. urea. | |
|---|---|---|---|---|
| | Reading V°. | Velocity constant. $k$ | Reading V°. | Velocity constant. $k$ |
| 0 min. | +49.9 | ..... | +49.9 | |
| 2 min. | 49.4 | 0.0016 | 49.6 | 0.0009 |
| 5 min. | 48.9 | 0.0013 | 49.4 | 0.0007 |
| 7 min. | 48.6 | 0.0012 | 49.3 | 0.0005 |
| 10 min. | 48.0 | 0.0012 | 49.1 | 0.0005 |
| 30 min. | 44.3 | 0.0013 | 47.2 | 0.0006 |
| 60 min. | 39.7 | 0.0012 | 44.8 | 0.0006 |
| 120 min. | 31.4 | 0.0012 | 40.1 | 0.0006 |
| 180 min. | 24.7 | 0.0012 | 35.8 | 0.0006 |
| 2 days | −16.5 | ..... | −17.2 | ..... |
| 4 days | −16.5 | ..... | −21.3 | ..... |
| Average | ..... | 0.00128 | ..... | 0.00063 |

Taking the reading before inversion as + 49.9 and the reading at completion of inversion as − 16.5 it is seen that the velocity of inversion $\left(k = \frac{1}{t}\log\frac{a}{a-x}\right.$, see p. 660$\left.\right)$, is diminished one-half by the addition of 5 gms. urea. There is no suspension of the inversion at the beginning, there being a decrease of 0.3 in the reading at the end of 2 minutes, and of 0.5 after 5 minutes. Under such circumstances it is impossible to take the true direct polarization.

A second objection to the Andrlik-Stanek modification is that the method cannot be used when reducing sugars are present owing to the change which the urea causes in their specific rotation. The extent of this change can be seen from the following experiments upon solutions of fructose, glucose, and invert sugar. The same volume of sugar solution was taken in each case and, after addition of substance, was completed to 100 c.c. The readings were taken immediately except as otherwise stated.

| | Fructose. | Glucose. | Invert sugar. |
|---|---|---|---|
| Volume completed with water alone..... | −26.2° V. | +56.5° V. | −10.2° V. |
| Volume completed with water + 5 gms. urea..... | −27.0 | +56.1 | −10.6 |
| Volume completed with water + 5 c.c. HCl | −26.9 | +56.7 | −10.5 |
| Volume completed with water + 5 gms. urea + 5 c.c. HCl..... | −27.3 | +56.5 | −10.7 |
| Volume completed with water + 5 gms. urea + 5 c.c. HCl after 2 days..... | −27.3 | +48.0 | −11.9 |

It is seen that the 5 gms. urea + 5 c.c. hydrochloric acid produce a different rotation than the 5 c.c. hydrochloric acid alone, this difference being greater for fructose. On long standing, glucose in presence of hydrochloric acid and urea shows a loss in rotation owing to the formation of glucose ureide ($[\alpha]_D = -23.5$). This explains the high levorotation of invert sugar solutions prepared in presence of urea. (See Table LII.)

The Andrlik-Stanek method is a dangerous one for it may introduce greater errors than those which it was designed to correct. The process, notwithstanding several favorable notices in the literature, is not to be generally recommended.

Among the modified methods belonging to Class II, which employ for the Clerget determination inverting agents less open to the objections of hydrochloric acid, may be mentioned the following:

(1) *Inversion by Means of Organic Acids.* — A number of organic acids, especially such as have no pronounced influence upon the optical activity of fructose, have been employed in place of hydrochloric acid for the determination of sucrose by the Clerget method. Weber* showed that in presence of acetic acid invert sugar had the same rotatory power as in aqueous solution. Acetic acid, however, is an unsatisfactory reagent for the Clerget determination on account of its very weak inverting action ($_2\frac{1}{5}\frac{1}{0}$ that of hydrochloric acid, see p. 663). Tolman† has tested the use of citric acid for the Clerget process and found that with 2 gms. of this acid to 100 c.c. complete inversion of sucrose could be accomplished in 30 minutes at the temperature of boiling water. Under these conditions the Clerget factor for the normal weight of sucrose was 141.95 and for the half-normal weight 141.49. Tolman noted, however, that the presence of soluble acetates greatly retarded the inverting action of citric acid and that the latter was consequently of no value as an inverting agent with products which required previous clarification with lead subacetate. This same objection would apply to many other organic acids. Another serious objection, as with hydrochloric acid, against the use of organic acids as inverting agents is the difference in optical activity of contaminating amino compounds in the solutions used for direct and invert polarization — asparagine, for example, being levorotatory in aqueous solution, but dextrorotatory in presence of strong acetic acid.

Oxalic acid‡ has also been recommended as an inverting agent, 2 gms. of the acid being used for 100 c.c. of solution. This acid has

* J. Am. Chem. Soc., **17**, 321.

† Bull. 73, U. S. Bur. of Chem., p. 69.

‡ Kulisch. Z. ang. chem. (1897), 45.

a much stronger inverting power than either acetic or citric acid, but is open to the same objections previously stated.

The employment of organic acids as inverting agents in the examination of impure sugar products has not been found upon the whole to be satisfactory.

(2) *Inversion by Means of Invertase.* — The employment of yeast as an inverting agent in the Clerget determination of sucrose was first indicated by Kjeldahl* in 1881. O'Sullivan and Tompson,† in 1891, and Ling and Baker‡ in 1898, extended the use of the method and more recently Ogilvie has applied it to the analysis of sugar-factory products. The yeast method of O'Sullivan and Tompson, as modified by Ogilvie,§ is as follows:

"Four times the normal sugar weight of the sample are transferred to a standardized 200-c.c. flask, defecated with the minimum amount of basic lead-acetate solution (sp. gr., 1.26), a little alumina cream added, then the liquid adjusted to bulk at standard temperature, well shaken, and filtered; 100 c.c. of the filtrate are measured by a standard pipette into a small beaker, sulphur dioxide passed in from a siphon of the liquefied gas till a faint smell is perceptible (all the lead thus being indicated to be precipitated), then the liquid transferred to a 200-c.c. flask, made up to the mark, and well mixed. Now sufficient calcium carbonate (dried) in fine powder to neutralize the excess of acidity, and a little recently ignited kieselguhr (to promote filtration) are added, after which filtration follows. In this way a normal solution is obtained, which is sufficiently clarified to give a distinct polarimetric reading, is free from lead and excess of acidity, and is therefore well suited for the invertase inversion.

"Fifty cubic centimeters of the solution, prepared in the manner just described, contained in a 100-c.c. flask, are raised in a constant-temperature bath to between 50° and 55° C., after which 0.5 gm. of washed brewery yeast and 2 drops of acetic acid are added and the temperature maintained as near 55° C. as possible for $4\frac{1}{2}$ to 5 hours. At the end of this time the liquid is cooled, and a little alumina cream or kieselguhr added to assist filtration, and made up to bulk at standard temperature. The clear filtrate is then polarized in a lateral-branched water-jacketed tube at exactly 20.0° C."

The Clerget factor determined by Ogilvie for the above process from experiments upon pure sucrose is 141.6.

* Compt. rend. Lab. Carlsberg (1881), **1,** 192. † J. Chem. Soc. Trans., **59,** 46.
‡ J. Soc. Chem. Ind., **17,** 111. § Int. Sugar Jour., **13,** 145.

Instead of employing yeast, a solution of invertase prepared therefrom may be used to advantage. Hudson* has developed a method upon this principle, which is described as follows:

" Dissolve 26 gms. of the substance to be analyzed for cane sugar in water, clarify with the usual substances (neutral or basic lead acetate or alumina cream or kaolin) and make up to 100 c.c. volume at 20° C. Filter and read the polarization of the filtrate in a 200-mm. tube. Remove the excess of lead from the filtrate, if lead has been used as clarifying agent, with sodium carbonate or potassium oxalate, and filter. To 50 c.c. of the filtrate add acetic acid by drops until the reaction is acid to litmus, add 5 c.c. of the stock invertase solution (p. 669), and make up the volume to 100 c.c. Add a few drops of toluene to the solution to prevent the growth of microörganisms, shaking so as to saturate, and allow to stand at any temperature between 20° and 40° C. over night. Under usual conditions about six hours' time is required to accomplish complete hydrolysis." When the inversion is finished, the solution is read at 20° C. and the invert reading calculated to the normal weight of substance. The Clerget factor for the above method as determined by Hudson from experiments upon pure sucrose is 141.7.

The invertase method is unquestionably the most ideally perfect of the numerous Clerget modifications. No disturbances are produced in the specific rotations of fructose, amino acids, or other optically active substances which may accompany sucrose and no other substances than sucrose are hydrolyzed except in the few special cases where raffinose, stachyose or gentianose may be present.

The complications involved in the preparation of the invertase reagent, the uncertainty of knowing whether a given preparation is always of constant strength, and the long period of time frequently necessary to accomplish inversion are the chief drawbacks against the use of the method in practical analytical work.

The inverting power of the stock invertase solution should be carefully determined from time to time by experiments upon pure sucrose and with any decrease in activity the quantity of reagent used for inversion must be correspondingly increased. The time of inversion can be shortened considerably by conducting the inversion at a temperature of about 55° C. To determine whether or not inversion is complete the closed flask or tube of solution may be warmed again to 55° C. for an hour and then, after cooling to 20° C., reread. If no change in polarization is noted, the inversion is complete.

* J. Ind. Eng. Chem., **2**, 143.

The invertase method will be found of especial value in research work and in controlling the results of other methods. In this connection, however, it should be noted that the influence of salts and other impurities upon the rotation of the accompanying sugars introduces the same error as in other Clerget modifications.

### Clarification of Solutions for the Determination of Sucrose by the Clerget Method

In the analysis of sucrose-containing products by the Clerget method, clarification by means of basic lead compounds must precede and not follow the process of inversion. This precaution is necessary, owing to the occlusion of a part of the invert sugar in the basic lead precipitate and the consequent diminution of the invert polarization. In so far as the work of analysis will permit, the solution for the direct polarization and that used for inversion should both be taken from the same clarified filtrate after deleading. The following method of procedure is given as an example.

**Method of Deleading.** — Transfer 57.20 gms. of product with about 100 c.c. of water to a graduated 200-c.c. flask. After solution, lead-subacetate reagent (1.26 sp. gr.) is added to the necessary point of clarification and the volume completed to 200 c.c. After mixing well, the solution is filtered and 100 c.c. of the filtrate (28.6 gms. substance) treated in a 110-c.c. flask with successive amounts of finely powdered potassium oxalate, or sodium carbonate, or sodium sulphate, etc., until no more lead is precipitated. If the deleaded solution is alkaline to litmus paper or phenolphthalein it is exactly neutralized with acetic acid and the volume completed to 110 c.c. The solution is mixed, filtered, and the filtrate (26 gms. substance to 100 c.c.) used for the direct polarization. Fifty cubic centimeters of the same filtrate are then inverted in a 100-c.c. flask, according to the method desired, and, after completing the volume to 100 c.c., polarized for the invert reading. The latter multiplied by 2 gives the invert polarization.

In this connection it should be remarked that with substances requiring large amounts of basic lead for clarification the 5 c.c. of hydrochloric acid prescribed for the Clerget or Herzfeld inversion may be insufficient on account of the formation of chlorides and the liberation of the weakly inverting acetic acid. In such cases it is usual to employ 6 c.c. of hydrochloric acid for making the inversion.

Instead of the powdered salts above mentioned, concentrated sulphurous acid (prepared by saturating water with sulphur dioxide) has been proposed by Pellet for deleading. This reagent has certain advantages,

for, in addition to precipitating excess of lead, it neutralizes any free alkalinity and at the same time acts as a bleach upon any coloring matter which might darken the solution for reading. The sulphur dioxide has even been added to excess for deleading, sufficient quantity (10 c.c.) of the solution being taken to complete the volume from 100 to 110 c.c. This excess does no harm, as the acid in the cold is a very weak inverting agent and has no immediate depressing influence upon the direct polarization. This excess of sulphurous acid has also the advantage of preventing the troublesome afterdarkening which frequently results from the inverting action of hydrochloric acid. Ogilvie* claims as another advantage an equalizing effect in the conditions before and after inversion in that both direct and invert polarizations are made in acid solution. It is evident, however, that the total quantity of acid is not the same in both cases and that these different amounts of acid will exercise a variable influence upon the rotation of fructose, amino compounds, etc.

An objection against sulphur dioxide as a deleading agent is the very troublesome character of the lead-sulphite precipitate which, on account of its finely divided colloidal condition, is very apt to pass through the filter. Agitating the solution with paper pulp, infusorial earth (kieselguhr), or kaolin previous to filtration has been recommended as a means of securing a clear filtrate.

**Decolorization of Inverted Solutions.** — The afterdarkening which results from the action of the hydrochloric acid upon coloring substances, caramel, or other organic impurities, is frequently so great as to cause difficulty in reading the solution for the invert polarization. In such cases a number of expedients may be followed.

(1) *Use of a* 100-*mm. or* 50-*mm. Tube.* — Since shortening the length of the observation tube always necessitates a corresponding multiplication of any errors of observation this method is to be used only as a last resort.

(2) *Decolorization by Means of Bone Black.* — Animal charcoal or bone black should never be used upon solutions for direct polarization on account of its great absorptive power for sucrose. It may, however, be employed with comparative safety upon solutions of invert sugar, provided the char be previously purified by washing with dilute hydrochloric acid and water and then dried. Two to five grams (depending upon the coloration of the solution) of the finely ground bone black are placed in the apex of a folded filter and the solution to be treated poured through in successive portions of about 10 c.c. The

* Int. Sugar Jour. **13,** 145.

first 25 to 30 c.c. of filtrate are discarded and the remainder used for the invert polarization.

(3) *Decolorization by Means of Reducing Agents, — Zinc Dust, Sodium Sulphite, Etc.* — A large number of reducing agents have been used for decolorizing acid solutions of invert sugar. Zinc dust has been frequently employed for this purpose, the destruction of coloring matter being due to the nascent hydrogen generated by the action of the hydrochloric acid upon zinc. The powdered metal is added to the solution to be decolorized in successive small amounts, thus preventing a too violent evolution of gas with loss of solution.

Sodium sulphite and bisulphite have also been employed for decolorizing acid invert sugar solutions. In this case the bleaching agent is the sulphur dioxide liberated by the action of the hydrochloric acid.

The use of zinc and sodium sulphite as decolorizing agents is not attended with serious danger, provided only the minimum amounts be employed.

*General Reliability of the Clerget Method*

While the method of double, or invert, polarization gives perfectly reliable results upon pure sucrose, it is evident that the method has serious limitations when applied to the investigation of impure products. The influence of mineral and organic impurities upon the specific rotations of sucrose and other sugars, and the lead-precipitate error affect all modifications of the Clerget process. The influence of hydrochloric acid upon the specific rotations of fructose and amino compounds is an additional source of error in all modifications where the invert polarization is made in hydrochloric acid solution. Under such circumstances the chemist need not expect, under the most favorable conditions, to obtain upon products containing a mixture of sucrose with reducing sugars, salts, and organic impurities an accuracy much greater than 0.5 per cent; in certain cases the error may exceed 1 per cent. The Clerget method gives therefore at best only an approximation, the degree of exactness depending not only upon the care and skill of the chemist, but also upon the nature of the substance being analyzed. The introduction of excessive refinements in the method has usually proved a thankless labor and is not to be recommended. The employment, for example, of a Clerget factor elaborated to the fifth decimal (as in Tuchschmid's formula, p. 264) is of no possible value in practical work.

In employing any of the numerous Clerget modifications it is always advisable for the chemist to establish his own factor for the

particular conditions of the analysis. This is best done by making a blank determination upon pure sucrose, or, better still, upon a mixture of pure sucrose with approximate amounts of the accompanying substances which are known to occur in the product undergoing examination. By so doing the chemist will gain an idea of the reliability of his method, such as can be secured in no other way.

### Application of the Clerget Method to the Determination of Sugars in Presence of Sucrose

When sucrose occurs in presence of another sugar, whose specific rotation is not affected by the inverting agent, and no other optically active substances are present, the percentage ($Z$) of the accompanying sugar may be determined as follows:

If $P$ is the direct polarization for the sucrose normal weight of substance, and $S$ the percentage of sucrose by the Clerget method, then $P - S$ is the polarizing power of the accompanying sugar. The percentage $Z$ may then be determined as upon page 200, by dividing the value $100\,(P - S)$ by the polarizing power of the accompanying sugar (Table XXXVI). The calculation may also be expressed in general terms by the equation

$$Z = \frac{66.5\,(P - S)}{[\alpha]_D^{20}},$$

in which 66.5 is the specific rotation of sucrose and $[\alpha]_D^{20}$ that of the accompanying sugar. The method of calculation may be illustrated by several examples.

*Example I.* — A sirup containing sucrose and dextrose gave a direct polarization of + 58.0 and an invert polarization of − 8.33 at 20° C. Required the percentages of sucrose and dextrose.

$$\text{Per cent sucrose} = \frac{100\,[58 - (-8.33)]}{142.66 - \frac{3.9}{2}} = \frac{6633}{132.66} = 50 \text{ per cent.}$$

$$\text{Per cent dextrose} = \frac{66.5(58 - 50)}{52.8} = 10 \text{ per cent.}$$

*Example II.* — A sirup containing sucrose and invert sugar gave a direct polarization of + 52 and an invert polarization of − 21 at 20° C. Required the percentages of sucrose and invert sugar.

$$\text{Per cent sucrose} = \frac{100\,[52 - (-21)]}{142.66 - \frac{3.9}{2}} = \frac{7300}{132.66} = 55 \text{ per cent.}$$

$$\text{Per cent invert sugar} = \frac{66.5\,(52 - 55)}{-20} = 10 \text{ per cent.}$$

*Example III.* — A sweetened condensed milk (26 gms. in 100 c.c.) gave a direct polarization of + 51.50 and after inversion in the cold a polarization of − 4.20 at 20° C. Required the percentages of sucrose and lactose.

$$\text{Per cent sucrose} = \frac{100\,[51.50 - (-4.20)]}{142.66 - \frac{2^0}{2}} = \frac{5570}{132.66} = 41.99 \text{ per cent.}$$

$$\text{Per cent lactose} = \frac{66.5\,(51.50 - 41.99)}{52.5} = 12.05 \text{ per cent.}$$

The percentages of sugars calculated in this manner have of course no greater degree of accuracy than the Clerget sucrose determination. With impure products clarified by means of basic lead compounds there may be an appreciable error due to the occlusion of reducing sugars in the lead precipitate.

**Method of Dubois for Determining Sucrose and Lactose in Milk Chocolate.** — Dubois* has applied the Clerget method to the determination of sucrose and lactose in milk chocolate. The usual procedure is somewhat modified in that 100 c.c. of water are added to the 26 gms. of substance, a correction being afterwards applied for the increase in volume through solution of sugars. A preliminary extraction of the chocolate with ether to remove fat secures a more rapid solution of sugars. The following method of solution may also be used.

Transfer 26 gms. of the finely ground chocolate to a flask, add 100 c.c. of water, cork and heat in a steam bath for 20 minutes, releasing the pressure occasionally during the first 5 minutes. Shake thoroughly twice during the heating so as to emulsify completely. Cool to room temperature, add 10 c.c. of lead-subacetate solution, mix and filter. After taking the direct polarization ($a$), delead the solution with dry potassium oxalate. Invert the deleaded solution according to Herzfeld's method and take the invert polarization ($b$), correcting for dilution. Calculate the approximate percentages of sucrose ($S$) and lactose ($L$) by the following formulæ:

$$S = \frac{(a - b) \times 110}{142.66 - \frac{t}{2}} \qquad L = \frac{(a \times 1.10) - S}{0.79}.$$

The approximate grams ($G$) of total sugar in the normal weight of chocolate are calculated from $S$ and $L$, and the volume ($X$) of solution estimated by the formula $X = 110 + (G \times 0.62)$, in which 0.62 is the increase in volume caused by dissolving 1 gm. of sugar in water. The corrected percentages of sucrose and lactose are then found as follows:

$$\text{True per cent sucrose} = \frac{SX}{110}. \quad \text{True per cent lactose} = \frac{LX}{110}.$$

* Cir. 66, U. S. Bur. of Chem., p. 15.

The employment of an expansion factor, as in the above method, is permissible only in case of water-free substances and where no other ingredients than sugars are dissolved. The factor 0.62 is not absolutely correct for all concentrations, as is seen from the following table:

| Sucrose dissolved in 100 c.c. water at 20° C. | Volume of resulting solution. | Increase in volume through solution of 1 gram sucrose. | Sucrose dissolved in 100 c.c. water at 20° C. | Volume of resulting solution. | Increase in volume through solution of 1 gram sucrose. |
|---|---|---|---|---|---|
| Grams. | c.c. | c.c. | Grams. | c.c. | c.c. |
| 1 | 100.51 | 0.506 | 26 | 115.98 | 0.614 |
| 2 | 101.12 | 0.560 | 50 | 130.94 | 0.619 |
| 5 | 102.96 | 0.592 | 100 | 162.37 | 0.624 |
| 10 | 106.07 | 0.607 | 200 | 225.82 | 0.629 |

The error attending the use of the factor 0.62 upon dilute solutions is so small as to be negligible.

### Application of the Clerget Principle to the Determination of Raffinose

The principle of the Clerget inversion method may be applied to the analysis of any optically active substance whose specific rotation undergoes a known change with a special method of treatment. The most common application of the principle, outside of sucrose, is in the determination of the trisaccharide raffinose, the occurrence of which in sugar-house products, plant substances, etc., is referred to on page 732.

The hydrolysis of raffinose with hydrochloric acid, under the conditions prescribed for the Clerget inversion, proceeds very closely according to the equation:

$$\underset{\substack{\text{Raffinose}\\ [\alpha]_D^{20}=+104.5 \text{ (for raffinose hydrate before hydrolysis)}}}{C_{18}H_{32}O_{16}} + H_2O = \underbrace{\underset{\substack{\text{d-Fructose}\\ [\alpha]_D^{20}=-92}}{C_6H_{12}O_6} + \underset{\substack{\text{Melibiose}\\ [\alpha]_D^{20}=+143}}{C_{12}H_{22}O_{11}}}_{[\alpha]_D^{20}=+53.5 \text{ (for raffinose hydrate after hydrolysis).}}$$

The specific rotation of raffinose decreases during the hydrolysis from +104.5 for the hydrate to +53.5, which corresponds to that of a molecular mixture of fructose and melibiose (see note p. 737). The normal weight of raffinose for the Ventzke scale, using metric cubic centimeters, is 16.545 gms. for the hydrate and 14.037 gms. for the anhydride (see p. 197). These amounts of raffinose, polarizing +100° V., show after hydrolysis, following exactly the procedure of Herzfeld, a polarization of + 51.24° V. at 20° C., or a decrease of 48.76° V. This decrease for the weight of raffinose reading 1° V. (0.16545 gm. hydrate

or 0.14037 gm. anhydride) is 0.4876° V. The calculation of raffinose by the hydrolysis method may then be expressed as follows:

$$R = \frac{P - P'}{0.4876},$$

in which $R$ is the percentage of raffinose, $P$ the polarization of the normal weight of product before hydrolysis and $P'$ the polarization of this normal weight after hydrolysis.

### Application of the Inversion Method to Mixtures of Sucrose and Raffinose

Raffinose is almost always associated in nature with sucrose, and since sucrose undergoes inversion simultaneously with the hydrolysis of raffinose, the formula previously given for the calculation of raffinose has but little practical value. Creydt,* however, showed that it was possible to combine the equations for the calculation of raffinose and sucrose, and in this way obtain formulæ which can serve for the estimation of the two sugars in mixtures. The original formulæ of Creydt were based upon the old Clerget process of inversion and have now been largely replaced by formulæ worked out for the Herzfeld† modification (p. 266). The method of establishing these formulæ may be understood from the following:

If the sucrose normal weight (26.00 gms.) of a substance containing $S$ per cent of sucrose and $R$ per cent of raffinose (anhydride) be dissolved to 100 metric cubic centimeters and polarized in a 200-mm. tube, the polarization of the sucrose in degrees Ventzke will be represented by $S$ and the polarization of the raffinose by 1.852 $R$ (the value 1.852 being the ratio $\frac{26.000}{14.037}$ of the normal weight for raffinose anhydride to that for sucrose). The direct polarization $P$ (the sum of the sucrose and raffinose polarizations) is represented then by the formula

$$P = S + 1.852\,R, \text{ whence } R = \frac{P - S}{1.852} \text{ and } S = P - 1.852\,R. \quad (1)$$

If the sucrose normal weight of the above substance be inverted according to the Herzfeld method and polarized at 20° C., the invert polarization of the sucrose will be represented by $-0.3266\,S$ (since 1° V. sucrose before inversion reads $-0.3266$° V. at 20° C. after inversion). In the same manner the polarization of the raffinose after hydrolysis will be $1.852\,R \times 0.5124 = 0.9490\,R$ (since 1° V. raffinose before hydrolysis reads $+0.5124$° V. at 20° C. after hydrolysis by Herzfeld's method).

* Z. Ver. Deut. Zuckerind., **37**, 153. † Ibid., **40**, 194.

The invert polarization $P'$ (the sum of the sucrose and raffinose invert polarizations) is represented then by the formula

$$P' = -0.3266\,S + 0.9490\,R. \qquad (2)$$

By substituting the quantity $\frac{P-S}{1.852}$ of equation (1) for $R$ in equation (2), we obtain the formula

$$P' = -0.3266\,S + \frac{0.9490\,(P - S)}{1.852},$$

whence

$$S = \frac{0.5124\,P - P'}{0.839}. \qquad (3)$$

Having calculated $S$ from $P$ and $P'$, the value of $R$ is obtained from equation (1), $R = \frac{P-S}{1.852}$.

By substituting the quantity $P - 1.852\,R$ of equation (1) for $S$ in equation (2), we obtain the formula

$$P' = -0.3266\,(P - 1.852\,R) + 0.9490\,R,$$

whence

$$R = \frac{0.3266\,P + P'}{1.554}. \qquad (4)$$

By formula (4) the raffinose may be calculated at once, from the direct and invert polarizations.

The method of employing the formulæ may be understood from the following:

A beet-molasses, free of reducing sugar, gave a direct polarization of $+50°$ V. and an invert polarization of $-12°$ V. Required the percentages of sucrose and raffinose.

By formula (3), per cent sucrose $= \frac{0.5124 \times 50 - (-12)}{0.839} = 44.84$ per cent.

By formula (1), per cent raffinose $= \frac{50 - 44.84}{1.852} = 2.79$ per cent, or

By formula (4), per cent raffinose $= \frac{0.3266 \times 50 + (-12)}{1.554} = 2.79$ per cent.

**Correction of Raffinose Formula for Changes in Temperature.** — The determinations of sucrose and raffinose by the preceding formulæ must be carried out at exactly 20° C. In case the analysis is made at other temperatures the formulæ require to be modified. Several formulæ have been worked out for correcting the invert polarizations of sucrose and raffinose for changes in temperature. Among the simplest of these are the formulæ of Herles,* which are derived as follows:

* Z. Zuckerind. Böhmen, **13,** 559; **15,** 528.

26.000 gms. of sucrose and 14.037 gms. of raffinose anhydride which read 100 per cent upon the saccharimeter before inversion, give after inverting by Herzfeld's method the following:

| Temperature. | Inverted sucrose solution. | Inverted raffinose solution |
|---|---|---|
| 20° C. | −32.66° V. | +51.24 |
| 0° C. | −42.66 | +47.24 |
| Difference for 20° C. | 10.00 | 4.00 |
| Difference for 1° C. | 0.50 | 0.20 |

For sucrose and raffinose reading 1 per cent upon the saccharimeter before inversion, the reading after inversion is:

For 1 per cent sucrose $= -0.4266$, at 0° C.
For 1 per cent sucrose $= -0.4266 + 0.005\,t$, at $t$° C.
For 1 per cent raffinose $= +0.4724$, at 0° C.
For 1 per cent raffinose $= +0.4724 + 0.002\,t$, at $t$° C.

The invert polarization for $S$ per cent sucrose

$$= S\,(-0.4266 + 0.005\,t).$$

The invert polarization for $R$ per cent raffinose

$$= R\,(+0.4724 + 0.002\,t),$$

or for the sucrose normal weight (26 gms.) $1.852\,R\,(+0.4724 + 0.002\,t)$.

The invert polarization $P'$ for $S$ per cent sucrose and $R$ per cent raffinose for 26.000 gms. to 100 c.c. would be

$$P' = S\,(-0.4266 + 0.005\,t) + 1.852\,R\,(+0.4724 + 0.002\,t).$$

Substituting for $R$ the value in equation (1), $R = \frac{P - S}{1.852}$.

$$P' = S\,(-0.4266 + 0.005\,t) + (P - S)\,(0.4724 + 0.002\,t).$$

Whence

$$S = \frac{P\,(0.4724 + 0.002\,t) - P'}{0.899 - 0.003\,t} \qquad (5)$$

and, as before, $R = \frac{P - S}{1.852}$. Equation (5) at 20° C. becomes necessarily the same as equation (3).

**Bone-black Error in Raffinose Determinations.** A source of error peculiar to certain applications of the inversion method for determining raffinose is the increase in levorotation after decolorizing inverted solutions by means of bone black. This error was first studied by Reinhardt,* who attributed the phenomenon to the ab-

* Z. Ver. Deut. Zuckerind., **52**, 114.

sorption of the highly dextrorotatory melibiose. Reinhardt's explanation is no doubt correct as bone black shows a similar absorptive power for other disaccharides, such as sucrose. Davoll,* who has made a detailed study of methods for estimating raffinose, gives the following results upon a mixture containing 94.98 per cent pure cane sugar and 5.02 per cent raffinose hydrate (4.26 per cent raffinose anhydride). The direct polarization for a normal weight of this mixture was + 102.48. The invert polarizations for different methods of treatment were as follows:

| Method of treatment. | Invert polarization. | Calculated sugars. | |
|---|---|---|---|
| | | Raffinose. | Sucrose. |
| | | Per cent. | Per cent. |
| Without char | −27.00 | 4.16 | 94.77 |
| Blood charcoal (purified with acid) | −27.14 | 4.11 | 94.87 |
| Animal charcoal (highest purity) | −27.40 | 3.95 | 95.16 |
| Animal charcoal (reagent) | −28.00 | 3.56 | 95.89 |

In the above experiments the solutions were shaken 5 minutes with 3 gms. of char before filtering. Pouring the solutions in successive portions through the char with rejection of the first runnings (as described on p. 220) would no doubt reduce the error due to absorption considerably.

As a remedy for the error due to the use of bone black Davoll proposes the employment of zinc dust as a decolorizing agent. At the end of the Clerget inversion 1 gm. of powdered zinc was allowed to act upon the acid solution at 69° C. for 3 to 4 minutes. Under these conditions the zinc was not found to affect the polarization of the inverted solution.

*General Reliability of the Optical Method for Estimating Raffinose*

The remarks (p. 278) made upon the limitations of the Clerget method apply with even greater force to the optical determination of raffinose. The method does not give accurate results, when optically active substances other than sucrose and raffinose are present. In cases where sucrose occurs with caramelization products, gums, and organic acids, application of the formula may indicate the presence of raffinose when in reality none is present. The formula should only be used in the investigation of substances in which raffinose is liable to occur (as sugar-beet products, cotton seed, etc.) and should never be

* Proc. Fifth Int. Congr. Applied Chem., III, p. 135.

employed, as is sometimes done, as a test for the presence of raffinose in unknown mixtures.

As in the Clerget determination of sucrose the chemist need not expect in the analysis of commercial products for raffinose an accuracy much exceeding 0.5 per cent. The indication of a smaller amount of raffinose than 0.5 per cent is, in fact, not regarded by the best authorities as sufficient to justify reporting its presence (as in raw beet sugars).

Before applying the method to the analysis of unknown products the chemist should first satisfy himself of the presence of raffinose by suitable tests (see p. 740); he should also confirm the results of his analysis so far as possible by making blank determinations upon known mixtures. A practical test of this kind is the best means for testing the reliability of the method in particular cases.

# CHAPTER XI

## SPECIAL METHODS OF SACCHARIMETRY

The methods of inversion, described in the previous chapter, are only special instances of a more general course of procedure. It is possible to calculate the percentage of any sugar, provided its rotatory power, in distinction from that of associated sugars, can be given a definite alteration by some special method of treatment. The changes produced in the rotation of sucrose and raffinose by the action of invertase or acids are but single illustrations of such special methods of treatment. As other examples may be mentioned (1) the determination of sugars by noting the change produced in polarization under different conditions of temperature. (2) The determination of sugars, by noting the change in polarization after fermenting with yeast. (3) The determination of sugars by noting the change in polarization after destroying the optical activity of reducing sugars. Numerous other examples might be given but the three cases cited are sufficient to illustrate the general application of the principle to special problems of saccharimetry.

### Determination of Sugars by Polarization at High Temperature

#### determination of invert sugar by high-temperature polarization

The principle of this method is based upon the fact that solutions of pure invert sugar, when heated to a temperature between 85° and 90° C., become optically inactive. This inactivity is due to the lowering in specific rotation of fructose with increase in temperature (page 179); the specific rotation of glucose being unaffected by temperature, the point of optical inactivity will be the degree at which the polarizing powers of glucose and fructose exactly neutralize each other.

**Temperature of Optical Inactivity of Invert Sugar.** — The temperature of optical inactivity of invert sugar has been variously estimated. Dubrunfaut,* who made the earliest measurements of this constant, set the figure at 90° C. Casamajor† and Wiley‡ have given 88° C.,

* Compt. rend., **42,** 901.
† Chem. News, **44,** 219.
‡ J. Am. Chem. Soc., **18,** 81.

Lippmann,* 87.8° C., Wolf,† 87.6° C. and Tuchschmid,‡ 87.2° C. These variations may be due in part to slight experimental errors (such as incipient destruction of sugar at the high temperature) and in part to the influence of concentration. Inasmuch as the $[\alpha]_D$ of glucose varies from + 52.5 for a 1 per cent solution to + 54.0 for a 40 per cent solution it is evident that the temperatures at which these different polarizations are neutralized must vary somewhat.

The effect of concentration upon the temperature of optical inactivity for invert sugar may be determined by means of the carefully established formulæ of Gubbe.‖

I Concentration $[\alpha]_D^{20} = -19.657 - 0.0361\ c.$

II Temperature

(20° to 100° C.) $[\alpha]_D^t = [\alpha]_D^{20} + 0.3246\ (t - 20) - 0.00021\ (t - 20)^2.$

In Table LIII, column *B* gives the $[\alpha]_D^{20}$ of invert sugar, as calculated by formula I, for different concentrations; column *C* gives the grams of invert sugar in 100 c.c. necessary to produce a reading of − 1° V., as calculated by the expression $\frac{1729}{100\,[\alpha]_D^{20}}$ (page 197); column *D* gives the temperature of optical inactivity, as determined by formula II of Gubbe; column *E* gives the variation in degrees Ventzke, produced by 1 gm. of invert sugar in 100 c.c. for 1° C. difference in temperature and is calculated by the expression $\frac{1}{C\,(D - 20)}$.

TABLE LIII.

| *A* | *B* | *C* | *D* | *E* |
|---|---|---|---|---|
| Concentration, grams invert sugar in 100 c.c. | $[\alpha]_D^{20}$ | Invert sugar in 100 c.c. corresponding to −1° V. at 20° C. | Temperature of optical inactivity. | Variation for 1 gram invert sugar for 1° C. |
| Grams. | | Grams. | Deg. C. | Deg. V. |
| 2 | −19.72 | 0.8768 | 83.2 | 0.01805 |
| 10 | −20.02 | 0.8636 | 84.2 | 0.01804 |
| 20 | −20.38 | 0.8484 | 85.4 | 0.01802 |
| 30 | −20.74 | 0.8336 | 86.6 | 0.01801 |
| 40 | −21.10 | 0.8194 | 87.8 | 0.01800 |
| 50 | −21.46 | 0.8057 | 89.0 | 0.01799 |
| 60 | −21.82 | 0.7924 | 90.2 | 0.01798 |

For general purposes 87° C. is usually taken as the temperature of optical inactivity for invert sugar.

* Ber., **13,** 1823.

† Oest. Ung. Z. Zuckerind., **15,** 331.

‡ J. prakt. Chem. [2], **2,** 235.

‖ Ber., **18,** 2207.

The application of the method to the determination of invert sugar is easily understood. Since a change of 1° C. produces a constant variation of 0.018° V. for 1 gm. of invert sugar in 100 c.c., regardless of the concentration, then the grams of invert sugar in 100 c.c. of a given solution is found by the formula

$$\text{Invert sugar} = \frac{P' - P}{0.018\,(t' - t)},$$

in which $P'$ = Ventzke-scale reading at higher temperature $t'$,
and $P$ = Ventzke-scale reading at lower temperature $t$.

The method of applying the formula may best be understood by taking a typical example.

*Example.* — 50 gms. of a solution, containing a mixture of glucose and fructose in unequal amounts, were made up to 100 c.c. at 20° C. The polarization was + 10.20° V. at 20° C. in a 200-mm. tube.

50 gms. of the same solution were made up to 100 c.c. at 87° C. The polarization was + 20.75° V. at 87° C. in a 200-mm. tube. Required the percentage of sugars in the original solution.

$$\text{Invert sugar} = \frac{20.75 - 10.2}{0.018\,(87 - 20)} = 8.75 \text{ gms.}$$

$$\frac{8.75}{50} \times 100 = 17.50 \text{ per cent invert sugar.}$$

The dextrorotation at 87° C. shows an excess of glucose over the amount necessary to be paired with the fructose for invert sugar. This excess of glucose may be estimated as follows:

Since 1° V. = 0.3225 gm. glucose (page 200) then the grams of glucose corresponding to the dextrorotation at the inactivity of invert sugar is 20.75 × 0.3225 = 6.69 gms. (uncorrected for concentration), or 13.38 per cent. To correct for the influence of concentration, the true glucose value of the Ventzke-scale reading + 20.75, according to the formula $G = s + 0.02\,s - 0.0002\,s^2$, (page 199) is 21.08. 21.08 × 0.3225 = 6.80 gms. glucose or 13.60 per cent in the original solution.

The percentage of glucose determined by this method of calculation can, of course, be considered as only approximate, for, as shown in Table LIII, the temperature of optical inactivity, according to concentration, may be above or below 87° C.

### DETERMINATION OF COMMERCIAL GLUCOSE BY HIGH-TEMPERATURE POLARIZATION

**Method of Chandler and Ricketts.** — The method of high-temperature polarization as first developed in 1880 by Chandler and Ricketts * was not employed for determining invert sugar but for detecting the presence and estimating the amount of commercial glucose in cane

* J. Am. Chem. Soc., **2**, 428.

sugar, molasses, honey and other products whose sugars, after inversion, consist almost wholly of invert sugar. The material under examination was first inverted to convert any sucrose to invert sugar and then polarized at the temperature of optical inactivity for invert sugar. Any dextrorotation observed at this temperature was attributed to commercial glucose and its percentage estimated by means of an empirical factor.

The factor for converting the readings of the Ventzke sugar scale into grams of commercial glucose depends entirely upon the nature of the product. Commercial glucose, as manufactured in the United States, varies in density from 41° Bé. to 45° Bé. (sp. gr. 1.388 to 1.442) and in specific rotation from about $[\alpha]_D + 100$ to $+ 125$ for the liquid product. The grams of commercial glucose corresponding to 1° V. for products of different specific rotation are given in Table LIV.

TABLE LIV.

| $[\alpha]_D$ (for liquid product). | Polarization (deg. V. of 26 grams to 100 true c.c.). | Grams of liquid product in 100 c.c. corresponding to a polarization of 1° V. | $[\alpha]_D$ (for liquid product). | Polarization (deg. V. of 26 grams to 100 true c.c.). | Grams of liquid product in 100 c.c. corresponding to a polarization of 1° V. |
|---|---|---|---|---|---|
| +125 | +188.0 | 0.1383 | +108 | +162.5 | 0.1600 |
| +120 | +180.5 | 0.1440 | +105 | +157.9 | 0.1647 |
| +115 | +172.9 | 0.1503 | +100 | +150.4 | 0.1729 |
| +110 | +165.4 | 0.1572 | | | |

For purposes of analysis the products of $[\alpha]_D + 108$ may be taken as the grade of commercial glucose most commonly used. The chemist should always state the polarizing power of the commercial glucose in terms of which his results are expressed.

The form of polariscope devised by Chandler and Ricketts for high-temperature polarization is shown in Fig. 146. The instrument consists of an ordinary saccharimeter, with trough removed and replaced by a water bath which is heated from below by means of gas or spirit lamps. The ends of the water bath, before the diaphragms of the analyzer and polarizer, are provided with metallic caps containing small windows of plate glass. The polarization tube, which in its earliest form was constructed of platinum, is completely immersed in the water of the bath, and rests upon supports opposite the windows and in perfect alignment with the axis of the instrument. The tube is provided with an upright tubule for inserting a thermometer and for receiving any excess of liquid displaced by expansion. The cover of the bath, which fits over the tubule, contains an opening for a thermometer to determine the temperature of the bath.

The use of a special type of saccharimeter for high-temperature polarization has been largely discontinued. At present it is customary to make the polarizations upon an ordinary type of saccharimeter, employing a metal-jacketed tube; the latter may be insulated to advantage by a mantle of asbestos or other non-conducting material. The

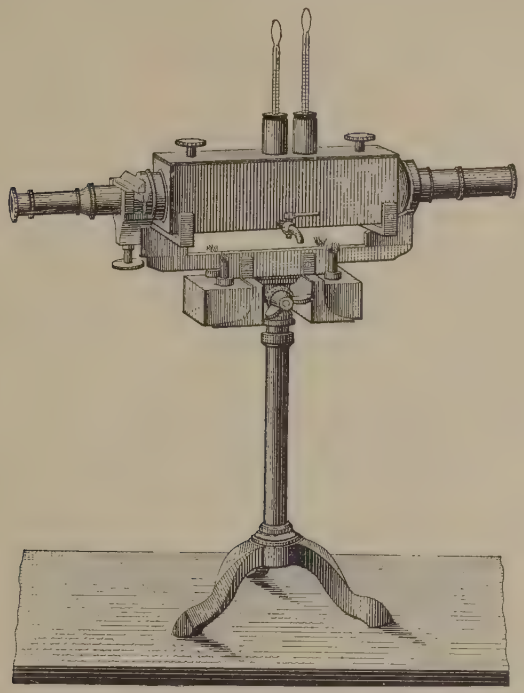


FIG. 146. — Chandler and Ricketts's polariscope for high-temperature polarization.

hot water for heating the tube is conveyed by rubber tubing from a water-heater, which should be placed at a distance sufficient to prevent heating the polariscope. A convenient arrangement for this purpose, described by Leach,* is shown in Fig. 147.

**Method of Leach.** — The following description of a method for determining commercial glucose in molasses, sirups, honey, etc., is given by Leach.†

* "Food Inspection and Analysis" (1911), p. 644.

† Bull. 81, U.S. Bur. of Chem., p. 73. Bull. 107 (revised), U.S. Bur. of Chem., p. 74.

"Invert a half-normal portion in the usual manner in a 100-c.c. flask; after inversion, cool, add a few drops of phenolphthalein and enough sodium hydroxide to neutralize; discharge the pink color with a few drops of dilute hydrochloric acid, add from 5 to 10 c.c. of alumina cream, and make up to the mark and filter. Multiply by 2 the reading at 87° C. in the 200-mm. tube; multiply this result by 100 and

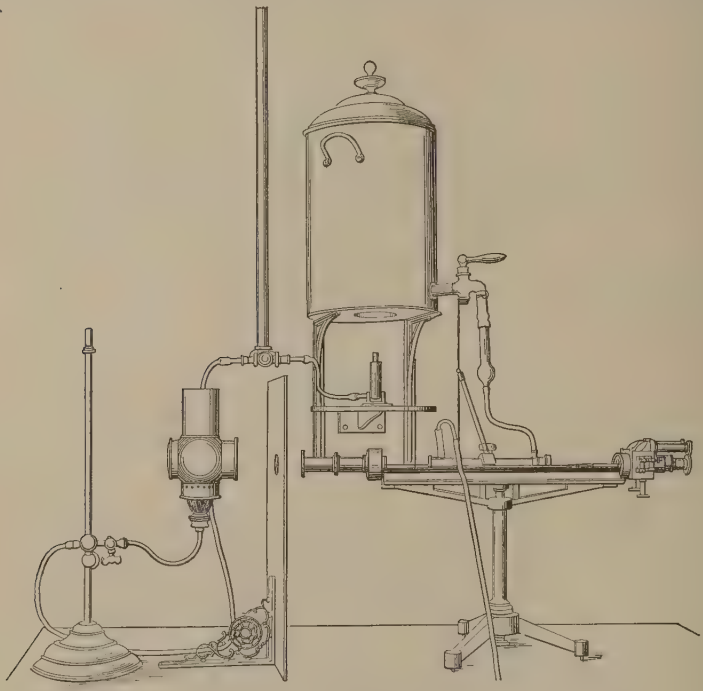


Fig. 147. — Apparatus for polarizing at high temperatures.

divide by the factor 163 to express the commercial glucose in terms of glucose polarizing + 175° V." *

In the above method the solution is made up at room temperature and polarized at 87° C. When this is done a correction must be made for the expansion of the solution and consequent lowering of the reading. The best method of making this correction is by means of an empirical test. Thus Lythgoe,† following the above course of

* Provisional Method of the Association of Official Agricultural Chemists, Bull. 107 (revised), U.S. Bur. of Chem., p. 71.

† Bull. 81, U.S. Bur. of Chem., p. 74.

procedure, obtained the following results upon five samples of commercial glucose.

| Sample. | Density. | Polarization (26 gms. in 100 c.c.). | | | Ratio $\frac{C}{B}$. | Ratio $\frac{C}{A}$. |
|---|---|---|---|---|---|---|
| | | *A* | *B* | *C* | | |
| | | Direct. | Invert at 22° C. | Invert at 87° C. | | |
| | Deg. Bé. | Deg. V. | Deg. V. | Deg. V. | | |
| 1 | 42 | 156.6 | 153.4 | 146.6 | 0.956 | 0.936 |
| 2 | 42 | 158.6 | 154.6 | 149.0 | .964 | .940 |
| 3 | 42 | 169.6 | 165.4 | 159.4 | .964 | .940 |
| 4 | 43 | 167.4 | 162.8 | 155.0 | .952 | .926 |
| 5 | 45 | 174.0 | 171.0 | 161.2 | .943 | .927 |
| | | | | Average | .956 | .933 |

It is seen that the polarization of commercial glucose is slightly lowered by the action of the acid during inversion, as well as by the expansion of the solution upon heating to 87° C. To correct for both of these influences, the polarization value of the glucose is multiplied by the factor 0.933. The Association of Official Agricultural Chemists expresses glucose in terms of a product polarizing 175° V. for a weight of 26 gms. in 100 c.c. and this polarization corrected gives $175 \times 0.933 = 163$ which is the factor employed in the calculation.

*Example.* — 13 gms. of a sample of table sirup inverted according to Herzfeld's method and made up to 100 c.c. at 20° C. polarized +65.2° V. at 87° C. Required the percentage of commercial glucose in terms of a product polarizing + 162.5° V. for 26 gms. in 100 c.c.

The factor for 162.5 is $162.5 \times 0.933 = 151.6$. Then $\frac{65.2}{151.6} \times 100 = 43.0$ per cent commercial glucose.

**Dextrorotation of Inverted Honey at 87° C.** — The method of estimating commercial glucose in honeys, sirups, molasses, etc., by polarizing at 87° C., can be regarded only as an approximate one. The chief limitation of the method is the fact that pure honeys, molasses, sirups, etc., are more or less dextrorotatory, after inversion, at 87° C., owing to the presence of gums, dextrins, or other similar compounds.

Table LV, which is taken from the work of Browne,* gives the polarization of various samples of American honey at 20° and 87° C., before and after inversion.

* "Chemical Analysis and Composition of American Honeys," Bul. 110; U. S. Bur. of Chem.

TABLE LV

| Kind of honey. | Number samples averaged. | Direct polarization. | | Invert polarization. | | Difference. |
|---|---|---|---|---|---|---|
| | | 20° C. | 87° C. | 20° C. | 87° C. | |
| | | Deg. V. | Deg. V. | Deg. V. | Deg. V. | |
| *Levorotatory Class:* | | | | | | |
| Mangrove | 1 | −24.80 | +0.50 | −27.94 | −0.66 | 27.28 |
| Mesquit | 3 | −20.93 | +4.45 | −25.01 | +2.83 | 27.84 |
| Sweet clover | 4 | −17.61 | +6.80 | −22.85 | +4.70 | 27.55 |
| Alfalfa | 8 | −15.10 | +9.63 | −22.99 | +5.00 | 27.99 |
| Buckwheat | 2 | −16.80 | +8.20 | −20.41 | +5.94 | 26.35 |
| Cotton | 2 | −17.50 | +6.80 | −21.01 | +6.05 | 27.06 |
| White clover | 15 | −13.01 | +11.65 | −17.77 | +9.25 | 27.02 |
| Goldenrod | 3 | −12.33 | +10.87 | −16.43 | +9.35 | 25.78 |
| Dandelion | 2 | −12.40 | +13.00 | −18.92 | +9.51 | 28.43 |
| Sumac | 3 | −10.47 | +12.53 | −14.01 | +11.51 | 25.52 |
| Apple | 2 | −8.55 | +17.00 | −13.73 | +12.76 | 26.49 |
| Basswood | 6 | −8.90 | +15.05 | −12.25 | +13.62 | 25.87 |
| Whitewood | 1 | −4.90 | +17.80 | −9.68 | +15.40 | 25.08 |
| *Dextrorotatory Class:* | | | | | | |
| Poplar | 1 | +3.60 | ..... | −2.53 | +20.90 | 23.43 |
| Hickory | 1 | +7.80 | +28.50 | +3.41 | +26.62 | 23.21 |
| White oak | 1 | +11.00 | +32.30 | +5.17 | +28.60 | 23.43 |
| Sugar-cane honey dew | 1 | +17.75 | ..... | +13.53 | +34.76 | 21.23 |
| Levorotatory honeys | 92 | −14.73 | +10.15 | −19.16 | +7.91 | 27.07 |
| Dextrorotatory honeys | 7 | +9.43 | +32.20 | +5.47 | +27.56 | 22.09 |
| Average of 50 varieties | 99 | −13.02 | +10.81 | −17.41 | +9.30 | 26.71 |

Application of the formula $\frac{100\,P}{163}$ to the invert polarizations at 87° C. would indicate nearly 10 per cent commercial glucose in some of the levorotatory and nearly 20 per cent in several of the dextrorotatory honeys.

**Browne's Method for Estimating Commercial Glucose in Honey.** — Browne* has modified the application of the high-temperature polarization, for estimating commercial glucose in honeys, by taking the difference between the invert polarization at 20° and 87° C. as a basis of calculation. It is seen from Table LV that while the invert readings at either 20° or 87° C. are subject to the widest variations, the difference between the polarizations at these two temperatures is a fairly constant quantity for nearly all honeys. The average value of this constant for the 99 samples of honey examined by Browne was 26.7. Since this difference in polarization is due entirely to the percentage of invert sugar in the honey, the addition of any commercial glucose will

* "Chemical Analysis and Composition of American Honeys," p. 60, Bul. 110; U. S. Bur. of Chem.

cause a depression in the polarization difference, which will be proportional to the amount of commercial glucose used but irrespective of its specific rotation. In order to correct for the variations in moisture and non-sugars of pure honey it is better to express the polarization difference in terms of a uniform basis of 77 per cent reducing sugars, which is the average percentage of invert sugar after inversion for pure honey. The formulæ for making the calculation are then:

$$\text{Per cent pure honey} = \frac{100(P' - P) \times 77}{26.7 \times I} = \frac{288.4\,(P' - P)}{I}$$

$$\text{Per cent commercial glucose} = 100 - \frac{288.4(P' - P)}{I}$$

in which $P'$ = the Ventzke polarization of the inverted honey at 87° C.
$P$ = the Ventzke polarization of the inverted honey at 20° C.
$I$ = the per cent of invert sugar in the honey after inversion.

Another method, used in European countries, for estimating the amount of commercial glucose in honey is based upon the variation in the invert polarization of the sample from that of pure honey. Calling the average invert polarization of pure honey − 17.5 at 20° C. (Table LV) and employing the official figure + 175° V. for the polarization of commercial glucose, then if

$x$ = per cent of honey in sample,
$y$ = per cent of commercial glucose in sample,
$P$ = invert polarization of sample in degrees Ventzke,

$$x + y = 100.$$
$$-0.175\,x + 1.75\,y = P$$
$$y = \frac{P + 17.5}{1.93}.$$

This method of calculation, the same as that based upon the polarization at 87° C., makes no allowance for the wide range in the invert polarization of individual honeys (−30 to + 15), so that a considerable error may be introduced in the final result.

In Table LVI the polarizations of 5 honeys and of mixtures of the same, with 20 per cent commercial glucose, are given together with the percentage of commercial glucose as calculated by the three methods described.

It will be seen from the results in the table that with admixtures of low-purity honeys and commercial glucose there is a considerable error in the calculation of the percentage of added adulterant. The results obtained by any method for estimating commercial glucose have only an approximate value, and in no case ought such analytical results as

those obtained for the pure basswood or white-oak honey to condemn a sample as being adulterated. In all suspicious or doubtful cases confirmatory qualitative tests such as that with iodine should be employed.

TABLE LVI *

*Polarization of Honeys and Commercial Glucose Mixtures, with Calculated Percentages of Glucose by Different Formulæ.*

| Kind of sample. | Direct polarization, 20° C. | Invert polarization. | | Polarization difference ($P'-P$). | Invert sugar after inversion, $I$. | Polarization difference corrected to 77 per cent invert sugar. | Calculated glucose. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $P$ 20° C. | $P'$ 87° C. | | | | $\frac{100\,P'}{163}$. | $\frac{P+17.5}{1.93}$. | $100-\frac{288.4(P'-P)}{I}$. |
| | Deg. V. | Deg. V. | Deg. V. | Deg. V. | Per cent. | Deg. V. | Per cent. | Per cent. | Per cent. |
| Alfalfa | −19.5 | −22.66 | + 3.52 | 26.18 | 77.84 | 25.90 | 2.16 | 0.00 | 3.00 |
| Alfalfa+20 per cent glucose | +19.4 | +16.88 | +35.82 | 18.94 | 70.01 | 20.83 | 21.97 | 17.82 | 21.98 |
| Hop vine | −12.6 | −16.83 | + 9.68 | 26.51 | 75.83 | 26.92 | 5.94 | .35 | .00 |
| Hop vine+20 per cent glucose | +24.9 | +21.54 | +40.74 | 19.20 | 68.14 | 21.70 | 25.00 | 20.28 | 18.72 |
| Whitewood | − 4.9 | − 9.68 | +15.40 | 25.08 | 71.88 | 26.87 | 9.45 | 4.06 | .00 |
| Whitewood+20 per cent glucose | +31.1 | +27.26 | +45.32 | 18.06 | 64.99 | 21.40 | 27.80 | 23.25 | 19.85 |
| Basswood | − .3 | − 1.32 | +23.21 | 24.53 | 70.60 | 26.75 | 14.24 | 8.40 | .00 |
| Basswood+20 per cent glucose | + 3.48 | +33.94 | +51.57 | 17.63 | 63.97 | 21.22 | 31.64 | 26.72 | 20.52 |
| White oak | +11.0 | + 5.17 | +28.60 | 23.43 | 70.44 | 25.61 | 17.56 | 11.23 | 4.08 |
| White oak+20 per cent glucose | +43.8 | +39.14 | +55.88 | 16.74 | 63.84 | 20.20 | 34.28 | 29.35 | 24.35 |

* " Chemical Analysis and Composition of American Honeys," Bul. 110, U. S. Bur. of Chem., p. 61.

**Dextrorotation of Inverted Molasses at 87° C.** — The observations made upon the dextrorotation of inverted honey at 87° C. also pertain to sugar-cane molasses and sirups, but to a much less degree. Eighteen samples of Louisiana sugar-cane molasses, of known purity, examined by Bryan,† gave an average direct polarization at 20° C. of + 40.6° V., an average invert polarization at 20° C. of −17.8° V. and an average invert polarization at 87° C. of + 2.53, the range of the latter being from 0.0 to + 4.18, or an equivalent of 0 to 2.5 per cent commercial glucose.

### DETERMINATION OF FRUCTOSE BY POLARIZATION AT LOW AND HIGH TEMPERATURES

**Method of Wiley.**— A second illustration of the methods of high-temperature polarization is afforded by Wiley's ‡ method for estimating fructose. In his description of this method Wiley shows that 1 gm. of fructose in 100 c.c. of solution gives a variation of 0.0357° V. for each 1° C. difference in temperature. The grams of fructose present in 100 c.c. of any solution can be calculated, therefore, from the polariza-

† Bull. 122, U. S. Bur. of Chem., p. 182.

‡ Wiley's "Agricultural Analysis" (1897), **3**, 267.

tions made at two widely separated temperatures by means of the formula.

$$F = \frac{P' - P}{0.0357(t' - t)},$$

in which $F$ = grams of fructose in 100 c.c. of solution.
$P'$ = Ventzke polarization at high temperature $t'$.
$P$ = Ventzke polarization at low temperature $t$.

The factor 0.0357 employed by Wiley is confirmed by the observations of other investigators as shown in Table LVII.

TABLE LVII.

*Showing Change of Polarization of Fructose for 1° C. Change of Temperature*

| Observer. | *A* Change in $[\alpha]_D$ of fructose per 1° C. | *B* Change in rotation for a fructose solution reading 100° V. per 1° C. $\frac{100\ A}{92.5}$. | *C* Change in rotation for 1 gram fructose in 100 c.c. per 1° C. $\frac{B}{18.692}$. |
|---|---|---|---|
| Dubrunfaut* | 0.62 | 0.6702 | 0.03586 |
| Hönig and Jesser† | 0.68 | 0.7351 | 0.03933 |
| Jungfleisch and Grimbert‡ | 0.56 | 0.6054 | 0.03239 |
| Gubbe§ | 0.63 | 0.6811 | 0.03644 |
| Tuchschmid \|\| | 0.64 | 0.6919 | 0.03702 |
| Average | 0.626 | 0.6767 | 0.03621 |

The average value 0.0362 is practically identical with that of Wiley.

Another method of determining the variation in the Ventzke polarization of fructose for changes in temperature is by means of Gubbe's equations (page 288). Since the specific rotation of glucose is not affected by changes in temperature, the results of Table LIII are converted into terms of fructose by dividing the values of columns $A$ and $C$, and by multiplying those of column $E$, by two. The variation in polarization of 1 gm. of fructose in 100 c.c. for 1° C. change in temperature, as thus determined, is 0.0360° V., which value is constant for all concentrations. This quantity, which is also the average of Wiley's figure and that of Table LVII, may be accepted as the most probable value.

* Compt. rend., **42,** 901.
† Z. Ver. Deut. Zuckerind., (1888), 1028.
‡ Compt. rend., **107,** 390.
§ Z. Ver. Deut. Zuckerind., **34,** 1345 ; calculated from results for invert sugar.
|| J. prakt. Chem. [2], **2,** 235; calculated from results for invert sugar.

If 26 gms. of product are made up to 100 c.c. and polarized ($P$) at a low temperature $t$, and a second 26 gms. are made up to 100 c.c. and polarized ($P'$) at a high temperature $t'$, then the percentage of fructose $F$ is determined by the equation

$$F = \frac{100\,(P' - P)}{26 \times 0.036\,(t' - t)} = \frac{100\,(P' - P)}{0.936\,(t' - t)}.$$

*Example.* — 26 gms. of honey made up to 100 c.c. and polarized at 20° C. gave a reading of − 14.8° V. 26 gms. of the same honey made up to 100 c.c. and polarized at 87° C. gave a reading of + 10.50° V. Required the percentage of fructose.

$$F = \frac{100\,[10.50 - (-14.8)]}{0.936\,(87 - 20)} = 40.34 \text{ per cent.}$$

In making polarizations at high temperatures it is desirable to make the readings as soon as the solution in the tube has reached temperature equilibrium, as indicated by the thermometer placed in the solution and by the disappearance of striations from the field. After noting the polarization the temperature is again taken and the average thermometer reading used in the calculation. Prolonged heating at high temperatures causes a destruction of fructose. A difficulty is sometimes experienced in obtaining a clear unobscured field of vision when using the hot-water polariscope tube. Too slow a circulation of hot water through the jacket of the tube, with production of currents of unequally heated solution, is the usual cause of the trouble. The hot water should be several degrees above the desired temperature and the circulation must be rapid enough to prevent loss of heat by radiation.

**Limitations of Methods of High-temperature Polarization.** — The method of determining invert sugar or fructose by polarization at widely-separated temperatures, while giving good results upon dilute solutions of the pure sugars, gives only an approximation in case of many sugar mixtures. The method is strictly applicable only when the specific rotations of the accompanying sugars are unaffected by changes in temperature; in all other cases there will be a certain error in the determination depending upon the temperature coefficient and the percentage of other sugars present. While no other sugars are affected to the same extent as fructose, yet it must be remembered that 1.5 gms. arabinose, or 3.0 gms. galactose, or 7.0 gms. maltose, or 9.0 gms. lactose, or 50 gms. sucrose produce approximately the same alteration in the Ventzke reading with 1° C. variation in temperature as 1 gm. of fructose, or 2 gms. of invert sugar.

But notwithstanding this limitation the method of high-temperature polarization has a distinctive value, and, when employed with due

caution, will be found of great service in many problems of analysis and research.

### Determination of Sugars by Polarization Before and After Fermentation

By employing pure cultures of specially selected organisms, it is sometimes possible to ferment one or more sugars of a given mixture, and from the variation in polarization thus produced to calculate the percentage of one or more of the members present.

**Action of Pure Yeast Cultures upon Different Sugars.** — The fermentative action of various yeasts upon different sugars has been studied by Tollens and Stone,* Hansen,† Fischer and Thierfelder,‡ and many others. The results of their experiments show a pronounced selective action on the part of different yeasts. While pure cultures of such well-known yeasts, as *Saccharomyces cerevisiæ*, or *Saccharomyces Pastorianus*, ferment completely d-glucose, d-fructose, d-mannose, d-galactose, sucrose, and maltose, these cultures are without action upon l-xylose, l-arabinose, rhamnose, sorbose and lactose. A "milk-sugar yeast," employed by Fischer and Thierfelder, fermented lactose and sucrose completely but did not attack maltose. *Saccharomyces apiculatus* ferments d-glucose, d-mannose and d-fructose but not galactose, sucrose, maltose or lactose. (See also Table CII, page 714.)

**Method of Fermentation.** — In carrying out experiments for the separation of sugars by fermentation it is very essential that the culture of particular yeast be pure. The presence of foreign yeasts, moulds or bacteria may produce changes in sugars, which a pure culture would leave unattacked. The solution to be fermented should be sterilized before inoculating.

The most favorable conditions for the action of the yeast are obtained with a solution containing about 10 per cent sugar and kept at a temperature of about 30° C. It is also necessary, in order to secure a rapid and complete fermentation, to have a suitable supply of nutritive matter present for the growth and sustenance of the yeast. A food supply for yeast in fermentation experiments is generally furnished by means of a nutritive salt solution or by means of yeast extract.

*Hayduck's Nutritive Salt Solution.* — Dissolve 25 gms. potassium phosphate, 8 gms. crystallized magnesium sulphate and 20 gms. asparagine in 1000 c.c. of spring water.

One cubic centimeter of the above solution to each 25 c.c. of liquid to be fermented insures a favorable development of yeast.

* Ann., **249**, 257. † Centralblatt, **88**, 1208, 1390. ‡ Ber., **27**, 2031.

*Yeast Extract.* — Wash 100 gms. of pure yeast (starch-free) repeatedly with cold water and repress. The residue of yeast is then heated to boiling for one-fourth hour with 500 c.c. of water; the liquid is then filtered through a folded filter, the filtrate, in case of turbidity, being returned to the filter until the extract runs through perfectly clear. The extract is then made faintly acid with citric acid, when it is sterilized and preserved in flasks closed by cotton wadding.

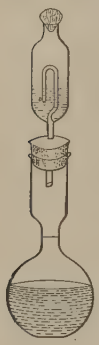


Fig. 148. — Fermentation flask.

The liquid to be fermented is diluted with an equal volume of the above extract.

Fermentation experiments are best carried out in flasks closed with a washing tube for the escape of carbon dioxide. The apparatus shown in Fig. 148 answers very well for the purpose. The fermentation is continued until bubbles of gas cease to pass through the water in the washing tube, when the process is considered to be finished. The washing tube is then removed, the solution heated to expel all carbon dioxide, and, after cooling, clarified, and the volume completed to the mark.

The polarization of the filtered solution is calculated to unfermented sugar, and the difference in polarization, before and after fermentation, calculated to fermented sugar. The application of the method is best understood from a special case.

*Example.* — By hydrolyzing a sample of sawdust with sulphuric acid, treating the resultant liquid with an excess of powdered calcium carbonate, filtering and evaporating, a sirup resulted which contained the two sugars, glucose and xylose.

50 gms. of the sirup, made up to 100 c.c., gave a polarization of + 43.5° V. in a 200-mm. tube.

50 gms. of the sirup were then diluted in a 200-c.c. flask with 100 c.c. of water and 5 c.c. of nutritive salt solution. After sterilizing, cooling and inoculating with pure-yeast culture, the flask was closed with a washing tube and fermented for 5 days in an incubator at 30° C. The evolution of gas having ceased, the solution was heated to expel $CO_2$, cooled, clarified with a little normal acetate of lead solution, made up to 200 c.c., and filtered. The polarization of the filtrate in a 400-mm. tube was + 5.2° V. Required the percentages of glucose and xylose in the sirup.

The loss in polarization by fermenting was 43.5 − 5.2 = 38.3° V. Since 1° V. = 0.3225 gms. glucose in 100 c.c. then the grams of glucose fermented were $38.3 \times 0.3225 = 12.35$ gms. or 24.7 per cent glucose (uncorrected) in the sirup.

Since 1° V.= 0.91 gms. xylose in 100 c.c., then, calling the residual

polarization of + 5.2 as due entirely to xylose, $5.2 \times 0.91 = 4.73$ gms. or 9.46 per cent xylose (uncorrected) in the sirup.

Corrections for concentration are made as indicated on page 198.

**Determination of Dextrin in Fruit Products.** — The fermentation method is sometimes employed for the determination of dextrin in jams, jellies and other products, which might be adulterated with commercial glucose. The provisional method of the Association of Official Agricultural Chemists is as follows:*

"Dissolve 10 gms. of the sample in a 100-c.c. flask, add 20 mgs. of potassium fluoride, and then about one-quarter of a cake of compressed yeast. Allow the fermentation to proceed below 25° C. for two or three hours to prevent excessive foaming, and then place in an incubator at a temperature of from 27° to 30° C. for five days. At the end of that time, clarify with lead subacetate and alumina cream, make up to 100 c.c. and polarize in a 200-mm. tube. A pure fruit jelly will show a rotation of not more than a few tenths of a degree either to the right or to the left. If a polariscope having the Ventzke scale be used and a 10 per cent solution be polarized in a 200-mm. tube, the number of degrees read on the sugar scale of the instrument multiplied by 0.875 will give the percentage of dextrin, or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}$$

in which

$C$ = degrees of circular rotation.
$L$ = length of tube in decimeters.
$W$ = weight of sample in 1 cubic centimeter."

The factor 0.875 is found as follows: Calling +198 the $[\alpha]_D$ of dextrin, then the grams of dextrin ($D$) in 100 c.c. of solution are found from the Ventzke reading ($V$) in a 200-mm. tube by the formula:

$$D = \frac{100(V \times 0.34657)}{2 \times 198} = .0875\ V.$$

If 10 gms. of product are made up to 100 c.c. then the percentage of dextrin in the sample $= \frac{.0875\ V}{10} \times 100 = 0.875\ V.$

The use of potassium fluoride in the method just described is to prevent the development of bacteria. Its employment is not necessary when pure-yeast cultures are used and the solution to be fermented has been previously sterilized.

* Bull., 107 (revised) U. S. Bur. of Chem., p. 80.

The work of Brown and Morris* shows that the dextrins and maltodextrins of starch conversion are not fermented by *Saccharomyces cerevisiæ;* their experiments prove, however, that other yeasts, such as *Saccharomyces ellipsoideus* and *Saccharomyces Pastorianus*, strongly ferment these dextrins. In carrying out the fermentation method for the estimation of dextrin, it is best to work with a pure culture of *Saccharomyces cerevisiæ.*

**Limitations of Fermentation Methods.** — The methods of estimating sugars by difference in polarization, before and after fermentation, give at best only a fair approximation. Several dangers attend the employment of the method, chief among which are the attack of sugars, or carbohydrates, supposed to be unfermented, and the incomplete destruction of sugars supposed to be completely fermented. Careful attention to the details of pure culture, sterilization and nutrition will, however, largely eliminate these dangers. The formation of optically active fermentation by-products may introduce a disturbing factor under certain irregular conditions, but with a normal alcoholic fermentation the error from this cause is insignificant. The optical activity of the nutritive solution used in the experiments should of course be determined, and its value, if significant, should be considered in the calculation.

The length of time required for completing a determination has been a strong objection against the use of fermentation methods in general sugar analysis. The more rapid, and generally more accurate, methods based upon polarizing and copper-reducing power have, for this reason, been given the preference.

### Polariscopic Methods Based on Destroying the Optical Activity of Reducing Sugars

The determination of sugars by methods of this class is based upon the fact that solutions of reducing sugars, when heated with alkalies or alkalies and hydrogen peroxide, or with alkalies and metallic oxides or salts, lose more or less completely their optical activity. These methods have been applied not so much to the determination of reducing sugars themselves, as to the determination of sucrose, dextrin and other non-reducing carbohydrates in presence of reducing sugars.

#### Destruction of Optical Activity of Reducing Sugars by Means of Alkalies

**Method of Dubrunfaut.** — The first efforts to establish a quantitative method in this direction were made by Dubrunfaut† in 1850.

* J. Chem. Soc. Trans., **47**, 527. † Compt. rend., **32**, 439.

Later investigators found, however, that the end-products in Dubrunfaut's method, obtained by the action of different alkalies upon reducing sugars, were not completely inactive, so that the polariscopic reading always required a certain correction. Efforts to establish a constant correction factor for modifications of Dubrunfaut's method have been made by Pellet,* Jesser,† Koydl,‡ Bardach and Silberstein§ and others, but the results, on account of the variability in conditions, have not been wholly satisfactory.

**Method of Lobry de Bruyn and van Ekenstein.** — The rate of destruction of optical activity upon heating solutions of reducing sugars with dilute alkalies is illustrated by the following experiment taken from the work of Lobry de Bruyn and van Ekenstein;‖ 20 gms. of anhydrous glucose were heated with 10 c.c. of normal potassium hydroxide in 500 c.c. of solution at 63° C. The following decrease in rotation was noted:

| Time. | Angular rotation. | Specific rotation. | Time. | Angular rotation. | Specific rotation. |
|---|---|---|---|---|---|
| Minutes. | | | Minutes. | | |
| 10 | +5° 30′ | $[\alpha]_D = +46$ | 50 | 1° 50′ | |
| 20 | 4° 20′ | | 85 | 0° 43′ | |
| 30 | 3° 10′ | | 135 | ± 0° 10′ | $[\alpha]_D = \pm 1$ |
| 40 | 2° 20′ | | | | |

At the end of the experiment the solution had not darkened perceptibly and the original reducing power had only slightly diminished.

*Explanation of Optical Inactivity Produced by Alkalies.* — The explanation of the change of an optically active into an optically inactive solution of reducing sugar by action of alkalies was first given by Lobry de Bruyn and van Ekenstein. In the experiment just quoted the optical inactivity of the solution is due not to a destruction of glucose, but to its partial conversion into mannose and fructose, the combined rotations of the mixture of sugars producing optical neutrality. In one experiment the authorities, just named, noted after heating with alkali a loss of 18 per cent in reducing power; the residue was estimated to consist of 49 per cent unchanged glucose, 5 per cent mannose and 28 per cent fructose; the calculated rotation of such a mixture would in fact be very nearly zero.

* Bull. assoc. chem. sucr. dist., **8,** 623.
† Oest. Ung. Z. Zuckerind., **27,** 35.
‡ Ibid., **29,** 381.
§ Z. Unters. Nahr. Genussm., **21,** 540.
‖ Rec. Trav. Pays-Bas, **14,** 156, 203; **16,** 262.

**Method of Jolles.** — Recent experiments by Jolles * upon arabinose, glucose, fructose, invert sugar, lactose and maltose show that these sugars in 1 to 2 per cent solution are rendered optically inactive by heating for 24 hours at 37° C. with $\frac{1}{100}$ normal sodium hydroxide while sucrose is completely unchanged by this treatment. Stronger solutions of reducing sugars than 2 per cent show usually a residual activity after the alkaline treatment; it is necessary, therefore, in Jolles's method to dilute solutions to 2 per cent reducing sugar before making the determination. With substances containing much reducing sugar such dilution necessarily involves a considerable multiplication of any errors in the polariscope reading.

**Method of Bardach and Silberstein.** — Bardach and Silberstein † have modified Jolles's method so as to include solutions of reducing sugar up to 5 per cent concentration. Their method of procedure is as follows:

Take 45 c.c. of the neutralized sugar solution and make up to 50 c.c. with normal sodium hydroxide, thus making the solution $\frac{1}{10}$ normal alkaline. The solution is then polarized and a measured volume placed in a small beaker (8 to 10 cm. high and 5 cm. diameter) and kept at 36° to 39° C. for 20 hours by means of a thermostat, the beaker remaining uncovered. The solution is then cooled, made up to the original volume and repolarized. The final polarization is corrected for residual activity by means of an empirical factor, which in case of glucose was found to be as follows:

TABLE LVIII

*Showing Change in Polarization of Glucose upon Warming with Dilute Alkali*

| Approximate percentage of glucose in solution. | Polarization value. | | Approximate percentage of glucose in solution. | Polarization value. | |
|---|---|---|---|---|---|
| | Before treatment. | After treatment. | | Before treatment. | After treatment. |
| 0.5 | +0.51 | −0.09 | 2.5 | +2.54 | −0.36 |
| 1 | +1.02 | −0.19 | 3 | +3.05 | −0.26 |
| 1 | +1.02 | −0.15 | 3 | +3.06 | −0.27 |
| 1.5 | +1.53 | −0.26 | 4 | +4.10 | −0.32 |
| 2 | +2.04 | −0.25 | 4 | +4.07 | −0.25 |
| 2 | +2.05 | −0.26 | 5 | +5.12 | −0.21 |

The loss in polarization, after treatment with alkali under the prescribed conditions, must be diminished, therefore, by about 0.25 to give the correct polarization value of glucose. So also the residual

* Z. Unters. Nahr. Genussm., **20**, 631. † Loc.cit.

polarization must be increased by 0.25 to give the correct polarization equivalent of the residual sucrose, or other non-reducing carbohydrate present.

It is evident that the chemist in employing such methods as the above must establish his own correction factor for the particular reducing sugar with which he is working. The lack of absolute uniformity of conditions in the analysis of impure sugar products, leaves the general reliability of such correction factors more or less in doubt.

### DESTRUCTION OF OPTICAL ACTIVITY OF REDUCING SUGARS BY MEANS OF ALKALI AND HYDROGEN PEROXIDE

Other chemicals have been used in connection with alkalies to promote the destruction of reducing sugars. Lemeland,* for example, has devised a method for destroying the optical activity of reducing sugars in presence of sucrose by means of alkali, manganese dioxide and hydrogen peroxide.

**Method of Pellet and Lemeland.** — Pellet and Lemeland † have recently proposed a method for the analysis of sugar-cane molasses, which is based upon destroying the optical activity of reducing sugars by means of alkali and hydrogen peroxide. The details of the method are as follows:

"Make a solution of the cane molasses that will contain at most 5 per cent of reducing sugars. Measure 50 c.c. of this solution into a 300-c.c. flask, add 7.5 c.c. of sodium hydroxide (36° Bé.), then 75 c.c. of hydrogen peroxide (12 vols.), and 60 c.c. of water. Mix and place the flask in a boiling water-bath for 20 minutes, cool, neutralize the remaining alkalinity fairly exactly with acetic acid, and defecate with basic lead-acetate solution (36° Bé.), the necessary amount of which will be found to vary from 15 to 40 c.c., according to the weight of the material taken, the amount of reducing sugars destroyed and the impurities initially contained in the liquid. Complete the volume to 300-c.c., mix well and filter. First polarize directly in the 200-mm. or 400-mm. tube. Then 50 c.c. of the filtered liquid may be taken, 1 c.c. of glacial acetic acid added to it, the volume completed to 55 c.c., and after mixing a second polarization made, account being taken of the dilution. This is done because the second polarization is often a little different from the first, in which the liquid is alkaline. If a difference is observed, then the second, or acid polarization, should be used. The percentage of sucrose is calculated on the solution, and then on the sample."

* J. Pharm. Chim., **2**, 298.

† Int. Sugar J., **13**, 616.

The authors state that the results by this method agree very closely with those obtained by the method of inversion, when special precautions are observed to insure the utmost accuracy.

### DESTRUCTION OF OPTICAL ACTIVITY OF REDUCING SUGARS BY MEANS OF ALKALI AND MERCURIC CYANIDE

**Method of Wiley.** — The destruction of the optical activity of reducing sugars by means of Knapp's alkali-mercuric-cyanide solution was first employed by Wiley* in the determination of dextrin in commercial glucose. The reagent is prepared as follows:

*Alkali-mercuric-cyanide Solution.* — Dissolve 120 gms. sodium hydroxide and 120 gms. mercuric cyanide in separate portions of water; the two solutions are then mixed and made up to 1000 c.c. Any precipitate which forms is removed by filtration.

In making the determination 10 gms. of the commercial glucose are dissolved in water and made up to 100 c.c.; 10 c.c. of this solution are transferred to a 50-c.c. graduated flask, 20 to 25 c.c. of the alkali-mercuric-cyanide solution are added, and the mixture boiled 3 minutes under a well-ventilated hood. The solution is cooled, and neutralized with concentrated hydrochloric acid, the latter being added until the brown color of the liquid is just discharged. The solution is then clarified, made up to volume, filtered and polarized. The optical activity of the maltose and dextrose being destroyed, the residual polarization is that of the dextrin.

In Wiley's experiments, the specific rotation of the dextrin was taken as + 193. Adopting this figure, and taking the reading of a Ventzke-scale saccharimeter, the grams of dextrin in 100 c.c. of solution $= \frac{66.5 \times 0.26}{193} V^\circ = 0.0896\ V^\circ$. Since the solution polarized contained 1 gm. of original sample in 50 c.c. (or 2 gms. in 100 c.c.), then $\frac{0.0896\ V^\circ}{2} \times 100 =$ per cent dextrin in the commercial glucose.

In concluding this chapter upon special methods of saccharimetry the chemist is advised, as in case of the methods of inversion, to test the reliability of any untried process by means of check analyses upon mixtures of known sugars. It is only in this way that an idea can be formed of the errors which are due to defect of method or to personal equation.

* Wiley's "Agricultural Analysis" (1897), **3**, 290.

# CHAPTER XII

## MISCELLANEOUS PHYSICAL METHODS AS APPLIED TO THE EXAMINATION OF SUGARS

In addition to specific gravity, refractive index and specific rotation there are a number of other physical constants, which, though of lesser analytical importance, have nevertheless a considerable value in certain investigations of sugars and sugar solutions. Among the constants of this class may be mentioned viscosity, heat of combustion, osmotic pressure, rate of diffusion, surface tension, heat of solution, thermal conductivity, specific heat and magnetic rotation. It is beyond the scope of the present volume to discuss the methods of making each one of these physical measurements. Viscosity, heat of combustion and the constants connected with osmotic pressure have acquired, however, a certain importance in general laboratory practice and the present chapter will discuss their use in the investigation of sugars.

### Viscosity of Sugar Solutions

The determination of viscosity is a measurement which is frequently applied to solutions of sugars and other carbohydrates for special purposes of technology, analysis or research. The viscosity of a liquid as ordinarily determined is an arbitrary constant and is usually taken as the ratio between times of flow, through a narrow tubular opening, of the same volumes of water and liquid, all conditions of temperature, etc., being the same.

Fig. 149.—Viscosity pipette.

**Viscosity Pipette.** — The simplest example of this method of measurement is afforded by the viscosity pipette. (Fig. 149.) The pipette is first filled with water so that its meniscus coincides with the upper mark *A;* after holding in a perfectly upright position the water is released and the interval of time noted for the passage of the meniscus from *A* to the lower mark *B*. The process is repeated a number of times and the average result taken as the water constant of the pipette at the temperature of the experiment. The pipette is dried and the process repeated in exactly the same manner with a sugar solution. If the average time of flow at 20° C. for water be 20.2

seconds and that of a sugar solution at 20° C. 105.1 seconds, then $\frac{105.1}{20.2} = 5.2$, the relative viscosity of the sugar solution at 20° C, as compared with water of the same temperature.

**Engler's Viscosimeter.** — The apparatus of Engler* (Fig. 150) is used very generally for determining viscosity. The instrument consists of a bath *B*, which is filled with water or oil of the desired temperature. The container *A* is gold plated, the conical bottom terminating

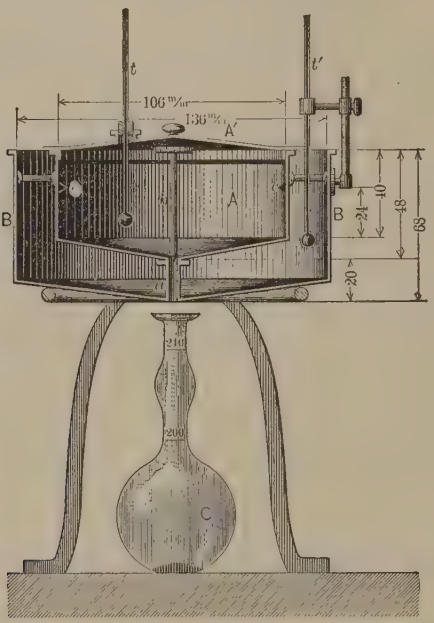



Fig. 150. — Engler's viscosimeter.

in a narrow tube *a*, 3 mm. wide and 20 mm. long, which serves as the outlet; the latter is closed by the valve rod *b*. The container holds at the marks *c* exactly 240 c.c. of solution. After filling to *c* with water or solution, the cover *A'*, holding a thermometer *t*, is placed in position and the temperature brought to the desired point. The valve rod is then withdrawn and the time noted for the delivery of exactly 200 c.c. of liquid in the flask *C*. The calculation of viscosity is made as previously described.

* König's "Untersuchung" (1898), p. 432.

**Coefficient of Viscosity.** — While the viscosity, as calculated by the above method, is sufficiently exact for many purposes, it is necessary in comparing liquids of different densities to employ the more exactly defined coefficient of viscosity.

In Fig. 151 the volume $V$ of liquid which is discharged in a time $t$ through a given capillary tube $A$-$B$ of the length $l$ and radius $r$ under a pressure $p$ is found by the equation

$$V = \frac{\pi \times p \times r^4 \times t}{8 \rho \times l}, \qquad (1)$$

in which $\rho$ is the coefficient of the interior friction of the liquid. It follows from the foregoing that

$$\rho = \frac{\pi p r^4 t}{8 V l}. \qquad (2)$$

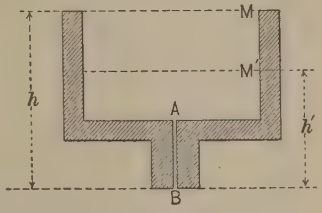



Fig. 151. — Showing principle of viscosimeter.

When $V$, $r$ and $l$ are unchanged, as happens in the use of the same viscosity apparatus, $\rho$ under constant pressure $p$ becomes

$$\rho = Kt, \qquad (3)$$

in which $K$ is a single constant peculiar to each individual viscosimeter.

In the previous figure the pressure $p$, with which a given volume of liquid $M$-$M'$ is discharged at the beginning of flow, is equal to its density $\delta$ multiplied by the height $h$ of its surface above the outlet $B$, and at the end of the flow to its density $\delta$ multiplied by the height $h'$. In the discharge of a constant volume $V$ of different liquids, between the marks $M$ and $M'$, $h$ and $h'$ are unchanged, so that for the mean pressure of flow, $p = C \times \delta$, in which $C$ is a constant. The coefficient of interior friction for different liquids using the same viscosimeter is then represented by the formula

$$\rho = K \times C \times \delta \times t,$$

in which $K$ and $C$ are two constants.

For water ($\delta = 1$), $\rho = K \times C \times t$. For any liquid of density $\delta$ and time of flow $\tau$, the viscosity coefficient $\eta$, or ratio between the internal friction of water and liquid, is

$$\eta = \frac{K \times C \times \delta \times \tau}{K \times C \times t} = \frac{\delta \tau}{t}.$$

The viscosity coefficients of liquids are, therefore, always proportional to the products of their densities and times of flow.

**Viscosity Coefficients of Pure Sucrose Solutions.** — The viscosity coefficients of pure sucrose solutions, as determined by Orth* for different concentrations and temperatures, are given in Table LIX.

TABLE LIX

*Viscosity Coefficients of Pure Sucrose Solutions*

| Grams sucrose in 100 grams solution. | Temperatures. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20° C. | 30° C. | 40° C. | 50° C. | 60° C. | 70° C. | 80° C. | 90° C. |
| 60 | 6.29 | 4.33 | 3.22 | 2.54 | 2.10 | 1.81 | 1.61 | 1.46 |
| 62 | 8.57 | 5.54 | 3.92 | 2.98 | 2.39 | 2.00 | 1.74 | 1.55 |
| 64 | 12.31 | 7.41 | 4.94 | 3.58 | 2.76 | 2.25 | 1.91 | 1.67 |
| 66 | 18.80 | 10.14 | 6.47 | 4.43 | 3.28 | 2.58 | 2.13 | 1.83 |
| 68 | 30.82 | 15.40 | 8.86 | 5.70 | 4.01 | 3.02 | 2.42 | 2.02 |
| 70 | 54.91 | 24.42 | 12.79 | 7.64 | 5.06 | 3.65 | 2.81 | 2.28 |
| 72 | 107.85 | 41.84 | 19.65 | 10.76 | 6.65 | 4.53 | 3.34 | 2.62 |
| 74 | 237.49 | 78.50 | 32.47 | 16.05 | 9.15 | 5.85 | 4.09 | 3.08 |
| 76 | 596.76 | 163.74 | 64.16 | 25.63 | 13.30 | 7.88 | 5.19 | 3.72 |

It is seen that at low temperatures the viscosity is much higher and that at certain concentrations it begins to undergo a most marked change in value. This relationship is made more plain in the opposite diagram (Fig. 152) which is taken from the work of Orth.

Attempts have been made to express the relationship between the viscosity and concentration of sugar solutions by means of a general equation. For dilute solutions the relationship according to Arrhenius † may be expressed by the equation

$$\eta = A^x$$

or

$$\log_e \eta = \log_e A(x),$$

in which $A$ is a constant and $x$ the concentration. According to this equation the natural logarithm of the viscosity coefficient is proportional to the concentration.

But for concentrated sugar solutions the above relationship does not hold. The law for solutions of high sucrose content, according to Orth, is expressed by the equation:

$$\eta = A^{(B^x)}$$

or

$$\log_e(\log_e \eta) = \log_e(\log_e A) + \log_e B(x)$$

in which $A$ and $B$ are constants.

* Bull. assoc. chim. sucr. dist., **29**, 137.

† Z. physik. Chem., **1**, 285.

For changes in temperature Orth gives the equation

$$\eta = A^{(B^x C^t)}$$

or $$\log_e(\log_e \eta) = \log_e(\log_e A) + \log_e B(x) + \log_e C(t);$$

in which $x$ and $t$ are the concentration and temperature of the sugar solution, and $A$, $B$ and $C$ constants.

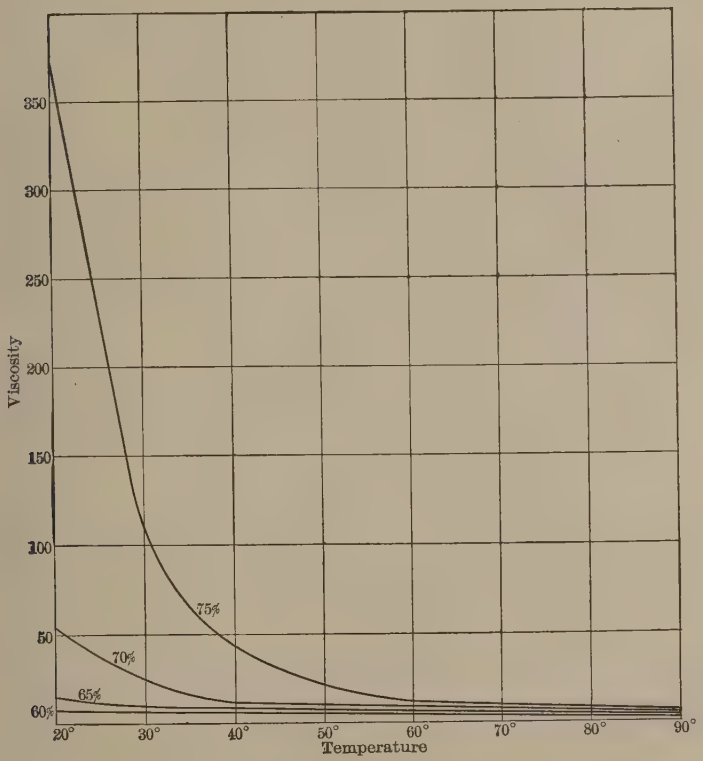



Fig. 152. — Diagram showing viscosity curves of four sugar solutions at different temperatures.

**Viscosity Coefficients of Impure Sucrose Solutions.** — From the viscosity coefficients of solutions of different sugar-house products Orth has made a compilation, the results of which are shown in Table LX.

TABLE LX

*Viscosity Coefficients of Sucrose Solutions of Different Purities.*

| Temperature. | Purity (per cent sucrose in solids). | Grams of solids in 100 grams of solution. | | | | |
|---|---|---|---|---|---|---|
| | | 65 | 70 | 75 | 80 | 85 |
| 20° | 100 | 15.09 | 54.91 | 369.67 | ..... | ..... |
| | 90 | 15.18 | 52.91 | 324.0 | 4450 | 196,600 |
| | 80 | 15.31 | 50.99 | 283.5 | 3251 | 102,960 |
| | 70 | 15.41 | 49.16 | 249.9 | 2400 | 55,360 |
| | 60 | 15.51 | 47.41 | 221.1 | 1808 | 30,770 |
| 40° | 100 | 5.62 | 12.79 | 43.03 | ..... | ..... |
| | 90 | 5.49 | 12.25 | 39.91 | 225.9 | 2,892 |
| | 80 | 5.35 | 11.74 | 36.96 | 199.4 | 2,334 |
| | 70 | 5.23 | 11.24 | 34.38 | 175.7 | 1,884 |
| | 60 | 5.10 | 10.78 | 32.03 | 155.3 | 1,538 |
| 60° | 100 | 3.00 | 5.06 | 10.95 | ..... | ..... |
| | 90 | 2.90 | 4.86 | 10.50 | 33.03 | 184.0 |
| | 80 | 2.81 | 4.67 | 10.05 | 31.97 | 183.2 |
| | 70 | 2.72 | 4.49 | 9.65 | 30.87 | 183.2 |
| | 60 | 2.64 | 4.33 | 9.27 | 29.90 | 183.2 |
| 80° | 100 | 2.01 | 2.81 | 4.59 | ..... | ..... |
| | 90 | 1.95 | 2.71 | 4.48 | 9.55 | 30.41 |
| | 80 | 1.89 | 2.63 | 4.37 | 9.65 | 33.24 |
| | 70 | 1.83 | 2.54 | 4.27 | 9.76 | 36.62 |
| | 60 | 1.78 | 2.47 | 4.17 | 9.84 | 40.33 |

The relation between viscosity and concentration of impure sugar-factory solutions is represented according to Orth by the equation

$$\eta = A(C^t)(B^{(x+Kn)}),$$

in which $t$ is the temperature and $K$ a linear function of $t$, $x$ the percentage of sucrose and $n$ the percentage of non-sugar, and $A$, $B$ and $C$ constants.

The viscosity of the non-sugars of sugar-house products was calculated by Orth not to differ greatly from that of pure sucrose; it was somewhat greater for the cold dilute and hot concentrated solutions and a little less for the other solutions, the average value for solutions of the same concentration being about 96 per cent that of sucrose.

The above conclusions of Orth pertain, however, only to the ordinary impurities of sugar-house products, such as reducing sugars, salts of mineral and organic acids, amino compounds, etc. The observation does not hold for dextran, levan and other gums which may occur in abnormal products and which greatly increase the viscosity of sugar solutions with consequent disturbance in the work of evaporating and boiling.

Excessive viscosities may also occur in sugar-house practice from supersaturation of sucrose, the result of careless sugar boiling. The successful sugar boiler aims to prevent supersaturation and to keep the viscosity of the pan contents as low as possible, in order that the maximum yield of sugar crystals may be obtained.

The determination of viscosity is of great value in certain branches of analytical work, as, for example, the examination of commercial dextrins, for which see page 508.

## Specific Heat of Combustion

**Units Employed in Calorimetery.** — The number of calories or heat units which a substance gives off, when burned in oxygen under specified conditions, is a constant which has been extensively used in the investigation of sugars. The determination has been especially employed in studying the calorific value of the different carbohydrates which are used in foods.

*The Small, or Gram, Calorie* (cal.) is defined as the quantity of heat necessary to raise 1 gm. of water through 1° C. The quantity of heat necessary to raise 1 gm. of water from 0° to 1° C. is not, however, exactly the same as that necessary to raise 1 gm. of water from 99° to 100° C., so that the measurement has been defined more precisely as one one-hundredth of the heat required to raise 1 gm. of water from 0° to 100° C.

*The Large, or Kilogram, Calorie* (Cal.) contains 1000 small calories, and may be defined, with the limitations previously noted, as the quantity of heat necessary to raise 1000 gms. of water through 1° C.

*The Centuple Calorie* (K) is defined as the quantity of heat necessary to raise 1 gm. of water from 0° to 100° C.

For ordinary purposes the ratio of the several units may be expressed as:

$$1\ \text{Cal} = 10\ \text{K} = 1000\ \text{cal.}$$

### The Bomb Calorimeter

The determination of calories of combustion is made in an atmosphere of compressed oxygen by means of a bomb calorimeter, the invention and extensive application of which to heat measurements are due to Berthelot.* The original bomb of Berthelot, on account of the large amount of platinum which it contains, is exceedingly expensive, and has been variously modified by Mahler, Hempel, Atwater and others for the purpose of reducing the cost. The Berthelot calorimeter,

* "Traité pratique de Calorimètrie chimique;" also Ann. chim. phys., [6] **6**, 546.

as modified by Hempel and Atwater* and improved by Blakeslee, is shown in Fig. 153.

**Description of Calorimeter.** — The most important feature of the calorimeter is the steel bomb, the cup ($A$) and cover ($B$) of which are lined with platinum, or heavily plated with gold. The cover is provided with a sunken lead gasket $K$, which rests upon the rim of the cup, and is held in place by the steel collar $C$, which is screwed tightly into position by means of a clamp and heavy spanner. The cover of the bomb is provided with a neck having an opening leading from $G$ to the interior of the bomb for the entrance of oxygen; the inlet is opened and closed by a valve screw $F$. The cover is also provided, on its inner surface, with two stiff platinum rods $I$ and $H$, between which passes a small spiral of iron wire for igniting the charge; the latter, consisting of 1 to 2 gms. of the sugar or carbohydrate to be burned, is placed in a platinum capsule, with a small piece of naphthalene to act as a kindler, directly under the spiral. The rod $I$ is connected through the cover with the electric wire $I'$ and the rod $H$, insulated from the cover, with the electric wire $H'$.

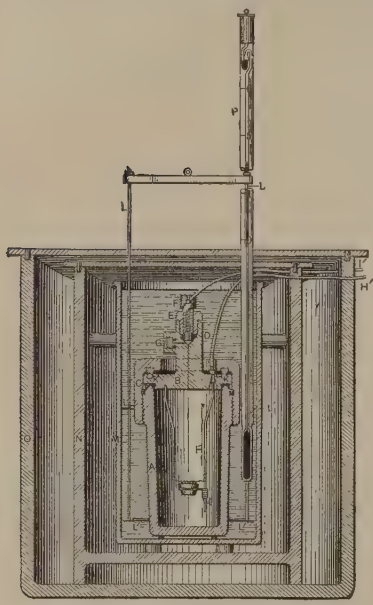


Fig. 153. — Bomb calorimeter.

**Operation of Calorimeter.** — The bomb, after introducing the charge, is filled with pure oxygen under 20 atmospheres pressure and then placed in the brittania-metal vessel $M$, which contains a weighed quantity of water, sufficient to cover all parts of the bomb. The vessel $M$ rests within two buckets, $N$ and $O$, which, with their covers, form two dead-air spaces, and insulate the bomb system from the room atmosphere. The temperature of the water in $M$ should be 2° to 3° C. below that of the inner air-chamber. A Beckmann thermometer, $P$, passes through the covers of the pails, and is fastened so that its

* See article by Atwater and Snell, J. Am. Chem. Soc., **25**, 659, for a very complete description of this instrument and its use.

bulb is immersed in the water about opposite the middle of the bomb. The thermometer can be read, by means of a magnifying lens, to the thousandth of a degree; it should be provided with a certificate for correcting errors of construction and for converting readings to true centigrade degrees. The mercury thread of the thermometer is adjusted at the desired point by partly filling or emptying the upper reservoir.

When the apparatus is in readiness the mechanical stirrer $L$ is set in motion and the thermometer read at intervals of one minute, tapping the top gently with an electric hammer before each reading to prevent lagging of the mercury thread. When five successive readings show a uniform rise in temperature, the electric switch is closed exactly at the end of the fifth minute. As soon as the extinction of the lamp in a resistance circuit indicates the fusion of the iron wire, the switch is reopened to avoid heating the water by the current. The readings of the thermometer should be noted at the end of each minute, until the maximum elevation of mercury is reached and the rate of fall has become regular. With the stirring mechanism making 40 revolutions per minute equilibrium is obtained usually within 5 minutes. After stirring 5 more minutes a final reading is taken, when the calculation may be made.

**Hydrothermal Value.** — The calories of combustion are calculated from the observations of a calorimeter experiment by multiplying the hydrothermal value (in grams) of the calorimeter system by the corrected rise in temperature and dividing the product (after subtracting the heat units due to accessory combustions) by the weight in grams of substance taken.

The accuracy of all calorimetric experiments is dependent upon the exactness with which the hydrothermal value of the calorimeter is known. The most common method for computing the water equivalent of the calorimeter system is to multiply the weight of each part by its specific heat and take the sum of these water equivalents as the hydrothermal value of the entire system. An example of the method is given by Fries, in Table LXI.

The hydrothermal value may also be determined by measuring the rise in temperature of the calorimeter system from burning a substance of known calorific value, as benzoic acid (1 gm. = 6322 cals.). For a description of this and other methods reference should be made to the work of Fries.*

* Fries, "Methods and Standards in Bomb Calorimetry," Bull. 124, Bur. of Animal Ind., U. S. Dept. of Agr., p. 9.

TABLE LXI

*Computed Water Value of Bomb Calorimeter*

| Material. | Weight. | Specific heats. | Water equivalent. |
|---|---|---|---|
| | Grams. | | Grams. |
| Steel | 3236.0 | 0.1114 | 360.49 |
| Platinum | 196.0 | 0.0320 | 6.27 |
| Lead | 66.0 | 0.0300 | 1.98 |
| German silver (approximate) | 4.0 | 0.0940 | 0.38 |
| Rubber (approximate) | 4.0 | 0.3310 | 1.32 |
| Iron (approximate) | 10.0 | 0.1114 | 1.11 |
| Mercury (approximate) | 50.0 | 0.0330 | 1.65 |
| Glass (approximate) | 10.0 | 0.1900 | 1.90 |
| Britannia metal | 855.0 | 0.0548 | 46.85 |
| Oxygen (constant volume) | 11.4 | 0.1570 | 1.79 |
| Water at 22° C. | 2000.0 | 0.9975 | 1995.00 |
| Total | | | 2418.74 |

**Correction for Radiation.** — When the conditions of the experiment are properly controlled the calorimeter system at the beginning of combustion is slightly cooler, and at the end of combustion slightly warmer, than the surrounding air. During the first period the calorimeter gains heat, and in the second loses heat to the surrounding air; the thermometer readings must be corrected, therefore, for the errors of radiation. This correction is made by the Regnault-Pfaundler* formula

$$C = nV + \frac{V' - V}{\theta' - \theta}\left(\frac{\theta_n + \theta_0}{2} + \sum_{1}^{n-1} \theta - n\theta\right),$$

where $n$ = number of time units (minutes) in combustion period. $V$ = rate of fall of temperature of calorimeter during initial period. (The change is actually a rise but for convenience is expressed as a fall, the value of $V$ thus being negative.)

$V'$ = rate of fall of temperature of calorimeter during final period.

$\theta$ = mean temperature of calorimeter during initial period.

$\theta'$ = mean temperature of calorimeter during final period.

$\theta_1, \theta_2, \ldots \theta_n$ = temperature at end of first, second, . . . $n$th minutes of combustion period.

$\theta_0$ = temperature at moment of ignition.

**Illustration of Method.** — The application of the formula is best understood from a special case and the following example of the combustion of sucrose is taken from a paper by Atwater and Snell.† The calorimeter employed had a water equivalent of 2100 gms. The data

* Pfaundler. Pogg. Ann., **129**, 113.

† J. Am. Chem. Soc., **25**, 659.

of the experiment are given in the following record, which is a convenient form for determinations of this kind.

Sample No. Description Cane Sugar. Date, July 13, 1901.
Bomb No. 3 Observer, J. F. Snell. Thermometer, No. 733.

Capsule No. 1
Wt. caps. + subs. = 4.2501
Wt. capsule = 2.8783
Wt. substance, $W$ = 1.3718

Correction for Accessory Combustions.
Wt. Fe 13.0 − 1.1 = 11.9 mgs. = 19.0 cal.
Wt. naphthalene = 6.4 mgs. = 61.6 cal.
$HNO_3$ = 6.6 cal.
Correction for accessories = 87.2 cal.

| | *Readings.* | *Corrected readings.* |
|---|---|---|
| Initial period. | 1 1.018 | 1.015 |
| | 2 1.021 | |
| | 3 1.025 | |
| | 4 1.027 | |
| | 5 1.030 | |
| | $6\,\theta_0$ 1.032 | 1.029 |
| Main period. | $7\,\theta_1$ 2.300 | 2.3 |
| | $8\,\theta_2$ 3.650 | 3.7 |
| | $9\,\theta_3$ 3.678 | 3.7 |
| | $10\,\theta_4$ 3.662 | 3.7 |
| | $11\,\theta_5$ 3.653 | 13.4 |
| Final period. | $\frac{\theta_5+\theta_0}{2}$ = 2.3 | |
| | Sum = 15.7 | |
| | $5\theta$ = 5.1 | |
| | Diff. = 10.6 | |
| | Log. diff. = 0253 | |
| | Log. $V'-V$ = 7324 | |
| | Colog. $\theta'-\theta$ = 5820 | |
| | 3397 | |
| | Antilog. = + .0219 | |
| | + 5 $V$ = − .014 | |
| | Radiation correction = + .0079 | |
| | 16 3.640 | 3.633 |
| | Time 3.30 | |

*Initial period.*

Fall = − .014
Rate $V$ = − .0028
Mean $t°, \theta$ = 1.022

*Corrected reading.*

$\theta_5$ = 3.646
$\theta_0$ = 1.029
$\theta_5+\theta_0$ = 4.675
$\frac{1}{2}$ = 2.3

*Final period.*

Fall = + .013
Rate $V'$ = + .0026
$V$ = − .0028
$V'-V$ = + .0054

Mean $t°, \theta'$ = 3.640
$\theta$ = 1.022
$\theta'-\theta$ = 2.618

*Thermometer correction.*

$T°$ air = 25.2
$T°$ water = 23.8
1st reading = 1.0
$T°$ of zero = 22.8
Corr. for 1° = + .001
Rise (degrees) = 2.6
Ther. corr. = + .0026

*Final calculations.*

$\theta_5$ = 3.646
$\theta_0$ = 1.029
$\theta_5-\theta_0$ = 2.617
Th. corr. = + .0026
Rad. corr. = + .0079
Corr. rise = 2.6275
Corr. rise × 2100 = total heat = 5517.8
Accessories = 87.2
Corrected heat = 5430.6
Log. corr. heat = 73485
Log. $W$ = 13729
59756
Heat of combustion per gram = 3959

Applying the formula to the above example, where the number of time units, $n$, is 5, we obtain for the several expressions, $V = -.0028$ and

$$nV = -.014;\quad \frac{V'-V}{\theta'-\theta} = \frac{+.0054}{2.618};\quad \frac{\theta_n+\theta_0}{2} = 2.3;\quad \sum_1^{n-1}\theta = 13.4;\ \text{and}\ n\theta = 5.1.$$

The combination of these values in the formula gives a radiation correction of $C = +0.0079°$.

The corrected rise of the Beckmann scale was 2.617 degrees and this corrected to true degrees C. and for radiation gives 2.6275° C. as the corrected rise in temperature, which, multiplied by 2100, the water equivalent of the calorimeter, gives 5517.8 calories.

*Correction for Accessory Combustions.* — The weight of the iron wire was 13 mgs. The quantity unburned was 1.1 mg. The quantity burned was therefore 11.9 mgs. The specific heat of combustion of iron being 1601 calories, the heat of combustion of 11.9 mgs. is $11.9 \times 1.6 = 19$ calories. The quantity of naphthalene burned was 6.4 mgs., which yields $6.4 \times 9.63 = 61.6$ calories, the specific heat of combustion of naphthalene being 9628 calories. The heat of combustion of nitrogen in the bomb as determined by titration of the nitric acid is 6.6 calories. ($N_2 + O_5 + H_2O = 2\ HNO_3$. 0.004406 gm. $HNO_3 = 1$ cal.) The total heat from accessory combustions is, therefore, $19 + 61.6 + 6.6 = 87.2$ calories.

Deducting this quantity from the total heat set free in the apparatus, we have $5517.8 - 87.2 = 5403.6$ calories as the heat due to the combustion of the sugar. The quantity of sugar burned was 1.3718 gms. The specific heat of combustion according to this determination is, therefore, $5430.6 \div 1.3718 = 3959$ calories.

**Gram-molecular Heat of Combustion.** — The gram-molecular heat of combustion is found by multiplying the calories per gram by the molecular weight ($M$). To avoid large figures it is customary to express this unit in terms of large calories.

$$\text{Gm. mol. Cals.} = \frac{\text{cals.} \times M}{1000}.$$

CALORIFIC CONSTANTS OF DIFFERENT SUGARS

In Table LXII, compiled by Tollens,* the calorific constants are given for the principal sugars, polysaccharides and sugar alcohols.

It is seen from the table that the molecular heat of combustion is always higher for the anhydride than for the hydrate of the same sugar. The molecular heat of combustion of the higher saccharides is also greater than the sum of the values of their components. Thus:

Sucrose = 1352.7 Gm. mol. Cals.

Glucose = 673.7
Fructose = 675.9
} = 1349.6 Gm. mol. Cals.

Difference = 3.1 Gm. mol. Cals.

This difference may be taken as the equivalent of heat which is liberated during inversion.

In the same way Raffinose = 2026.1 Gm. mol. Cals.

Glucose = 673.7
Fructose = 675.9
Galactose = 669.9
} = 2019.5 Gm. mol. Cals.

Difference = 6.6 Gm. mol. Cals.

* Tollens's "Handbuch der Kohlenhydrate," II, p. 45.

TABLE LXII.

*Giving Heats of Combustion of Sugars, Polysaccharides and Sugar Alcohols.*

| | cal. 1 gram. | Cal. (1 Cal. = 1000 cal.) for 1 gram-molecule. |
|---|---|---|
| *Sugars* | | |
| Arabinose, $C_5H_{10}O_5$ | 3722 (St.)<br>3714 (B.) | 558.3 (St.)<br>557.1 (B.) |
| Xylose, $C_5H_{10}O_5$ | 3746 (St.)<br>3740 (B.) | 561.9 (St.)<br>560.7 (B.) |
| Rhamnose, $C_6H_{12}O_5$ | 4379.3 (St.) | 718.5 (St.) |
| Rhamnose (cryst.), $C_6H_{12}O_5+H_2O$ | 3909.2 (St.) | 711.8 (St.) |
| Fucose, $C_6H_{12}O_5$ | 4340.9 (St.) | 712.2 (St.) |
| Glucose, $C_6H_{12}O_6$ | 3742.6 (St.) | 673.7 (St.)<br>677.2 (B.) |
| Galactose, $C_6H_{12}O_6$ | 3721.5 (St.) | 669.9 (St.) |
| Fructose, $C_6H_{12}O_6$ | 3755 (St.) | 675.9 (St.) |
| Sorbose, $C_6H_{12}O_6$ | 3714.5 (St.) | 668.6 (St.) |
| Sucrose, $C_{12}H_{22}O_{11}$ | 3955.2 (St.) | 1352.7 (St.) |
| Lactose, $C_{12}H_{22}O_{11}$ | 3951.5 (St.) | |
| Lactose, $C_{12}H_{22}O_{11}+H_2O$ | 3736.8 (St.) | 1345.2 (St.)<br>1340.6 (Gibson) |
| Maltose, $C_{12}H_{22}O_{11}$ | 3949.3 (St.) | 1350.7 (St.) |
| Maltose, $C_{12}H_{22}O_{11}+H_2O$ | 3721.8 (St.) | 1339.8 (St.) |
| Trehalose (anhydr.), $C_{12}H_{22}O_{11}$ | 3947.0 (St.) | 1349.9 (St.) |
| Trehalose (cryst.), $C_{12}H_{22}O_{11}+2\,H_2O$ | 3550.3 (St.) | 1345.3 (St.) |
| Raffinose (anhydr.), $C_{18}H_{32}O_{16}$ | 4020.8 (St.)<br>4020 (B.) | 2026.5 (St.)<br>2026.1 (B.) |
| Raffinose (cryst.), $C_{18}H_{32}O_{16}+5\,H_2O$ | 3400.2 (St.) | 2019.7 (St.) |
| Melezitose, $C_{18}H_{32}O_{16}+H_2O$ | 3913.7 (St.) | 2043.0 (St.) |
| *Polysaccharides:* | | |
| Cellulose, $(C_6H_{10}O_5)_n$ | 4185.4 (St.) | 678.0 (St.)<br>673.1 (Gottlieb)<br>680.4 (B.) |
| Starch, $(C_6H_{10}O_5)_n$ | 4182.5 (St.) | 677.5 (St.)<br>675.6 (Gibson) |
| Dextran, $(C_6H_{10}O_5)_n$ | 4112.3 (St.) | 666.2 (St.) |
| Inulin, $C_{36}H_{62}O_{31}$ | 4133.5 (St.) | 4092.1 (St.) |
| Glycogen, $(C_6H_{10}O_5)_n$ | 4190.6 (St.) | 678.9 (St.) |
| *Sugar Alcohols:* | | |
| Erythrite, $C_4H_{10}O_4$ | 4132.3 (St.) | 504.1 (St.)<br>502 (Louguinine)<br>502.6 (B.) |
| Arabite, $C_5H_{12}O_5$ | 4024.6 (St.) | 612.0 (St.) |
| Mannite, $C_6H_{14}O_6$ | 3997.8 (St.) | 729.9 (St.)<br>720.5 (Gibson) |
| Dulcite, $C_6H_{14}O_6$ | 3975.9 (St.) | 723.9 (St.) |
| Perseite, $C_7H_{16}O_7$ | 3942.5 (St.) | 836.1 (St.) |
| Quercite, $C_6H_{12}O_5$ | 4293.6 (St.) | 704.4 (St.)<br>710.4 (B.) |
| Inosite, $C_6H_{12}O_6$ | 3679.6 (St.) | 662.3 (St.)<br>665.5 (St.) |

St. = Stohmann and Langbein, J. prakt. chem. [2], **45**, 305.
B. = Berthelot and coworkers, from results in the Ann. chim. phys. [6], **6**, 552; **10**, 455; **13**, 304, 341; **21**, 409.

The hydrolysis of sugars may be regarded, therefore, as an exothermic reaction.

**Calculation of Calories from Chemical Formulæ.** — Various methods have been proposed for calculating the molecular heat of combustion from the chemical formula of sugars.

The calorific value for the combustion of the elements carbon (diamond) and hydrogen have been determined as follows:

$$C + O_2 = CO_2 + 94.3 \text{ Cals.}$$
$$H_2 + O = H_2O + 68.3 \text{ Cals.}$$

Welter's* rule for computing the molecular heat of combustion is to subtract as much O and $H_2$ as will unite to form water from the molecular formula, and multiply the number of remaining atoms by their respective heat values. The sum of the products is taken as the molecular heat of combustion.

*Example.* — Glucose $C_6H_{12}O_6$. The 6 atoms of O unite with 12 atoms of H to form 6 $H_2O$. The Cals. of the 6 remaining C atoms $= 6 \times 94.3 = 565.8$ Cals. This value is 16 per cent less than the value found experimentally by Stohmann, viz. 673.7 Cals.

A second method of calculating heat of combustion is to combine all the O and C that will unite to form $CO_2$, and calculate the heat of the remaining atoms in the manner just described.

To take again the example of glucose: The 6 atoms of O unite with 3 atoms of C to form 3 $CO_2$. The remaining $C_3$ and $H_{12}$ then give

For C, $3 \times 94.3 = 282.9$ Cals.
For $H_2$, $6 \times 68.3 = 409.8$ Cals.
692.7 Cals.

The results by this method are much closer than those obtained by Welter's rule, being about 3 per cent higher than the value found experimentally by Stohmann.

A third method of calculating heat of combustion is to distribute the O of the molecule among its C and H atoms according to the proportionate number and combining powers of the latter. Since the O necessary to form $CO_2$ is represented by 2 C and the O to form $H_2O$ by $\frac{H}{2}$, the uncombined equivalents of C and H, after deducting $CO_2$ and $H_2O$, would equal $2C + \frac{H}{2} - O$. The ratio of total to uncombined

* Walker's "Introduction to Physical Chemistry," (3rd Ed.), p. 129.

equivalents is then $\left(2\,C + \frac{H}{2} - O\right) \div \left(2\,C + \frac{H}{2}\right)$. The formula for the calculation is then:

$$\text{Gm. mol. Cals.} = \left(94.3\,C + 68.3\,\frac{H}{2}\right)\frac{2\,C + \frac{H}{2} - O}{2\,C + \frac{H}{2}}.$$

Applying this formula to glucose, we obtain,

$$\text{Gm. mol. Cals.} = \left(94.3 \times 6 + 68.3 \times \frac{12}{2}\right)\frac{12 + \frac{12}{2} - 6}{12 + \frac{12}{2}} = 650.4,$$

a result a little over 3 per cent below the value found experimentally by Stohmann.

The true molecular heat of combustion is about midway between the values calculated by the last two methods. It is evident, however, that absolute agreement cannot be attained by any method of calculation, since the experimental results are different for different isomers. The gram-molecule Calories for the $C_6H_{12}O_6$ sugars were found by Stohmann to vary from 668.6 for sorbose to 675.9 for fructose.

### Osmotic Pressure and Related Physical Constants, and Their Application in Determining Molecular Weights of Sugars

The determination of the molecular weights of sugars and sugar derivatives is a problem which may confront the chemist in his examination of unknown carbohydrates of plant or animal origin.

In the case of a reducing sugar an elementary analysis of one of its osazones or hydrazones (p. 370) will serve to fix the class to which the sugar belongs and thus indicate the molecular weight. In the case, however, of non-reducing sugars, such as sucrose, raffinose, etc., and of the sugar derivatives, which do not form osazones and hydrazones, a determination of the molecular weight by some physical method is usually required.

The molecular weights of sugar derivatives, which can be distilled without decomposition or dissociation, are best determined by the well-known vapor-density method of Victor Meyer. All the sugars, however, and most of their compounds undergo decomposition at or below the melting point so that the vapor-density method is excluded. Recourse is, therefore, usually made to some one of the methods which involve the principle of osmotic pressure.

### OSMOTIC PRESSURE OF SUGAR SOLUTIONS

Pfeffer,* the plant physiologist, in 1877, during his classical studies upon osmosis in vegetable cells, discovered that the osmotic pressure of dilute sugar solutions was proportional to the concentration. Pfeffer's experiments were performed by placing the sugar solutions in a porous bulb, which had deposited within its walls a semipermeable membrane of copper ferrocyanide. The bulb, which was connected with an upright tube, was then immersed in distilled water. The membrane, which is permeable to water but not to sugar, allows water to enter the bulb; the sugar solution begins to rise in the tube and the elevation continues until, after many hours, a maximum is reached; at this point the difference between the level of liquids within and without the bulb gives a pressure corresponding to the osmotic pressure of the sugar solution. This maximum pressure, expressed in centimeters or millimeters of mercury, was called by Pfeffer the osmotic pressure.

The following results by Pfeffer give the osmotic pressure of sucrose solutions at different concentrations.

| Concentration ($C$) of sucrose solution. | Pressure ($P$) in centimeters of mercury. | Ratio $\frac{P}{C}$. |
|---|---|---|
| Per cent. | | |
| 1 | 53.5 | 53.5 |
| 2 | 101.6 | 50.8 |
| 4 | 208.2 | 52.1 |
| 6 | 307.5 | 51.3 |

The ratio $\frac{P}{C}$ is a constant, the slight differences noted being due to variations in temperature and other experimental errors.

Pfeffer also showed that the osmotic pressure of sugar solutions underwent a regular increase with elevation of temperature. The following experiment was made upon a 1 per cent sucrose solution.

| Temperature C°. | Absolute temperature ($T$). | Osmotic pressure ($P$). | Ratio $\frac{P}{T}$. |
|---|---|---|---|
| 14.15 | 287.15 | 51.0 | .1776 |
| 15.5 | 288.5 | 52.05 | .1804 |
| 32.0 | 305.0 | 54.4 | .1784 |
| 36.0 | 309.0 | 56.7 | .1835 |

* Pfeffer's "Osmotische Untersuchungen," Leipzig, 1877.

The ratio $\frac{P}{T}$ is thus also found to be constant, the slight variations being due as before to experimental errors.

**Relation of Osmotic to Gas Pressure.** — In 1887 van't Hoff * showed that Pfeffer's osmotic pressures were identical in value with those obtained by gas pressure; in other words that the osmotic pressure per gram-molecule of substance is the same as the gas pressure per gram molecule at the same temperature and volume. This identity is expressed by the equation

$$pv = RT,$$

in which $p$ is the pressure and $v$ the volume, $T$ the absolute temperature and $R$ a constant. Van't Hoff showed that the constant $R$ is the same for substances in dilute solution as well as in the gaseous state.

The molecular weight of a substance is equal to the weight of its vapor in grams which would occupy the same volume, under equal temperature and pressure, as 2 grams of hydrogen (2 being the weight of the hydrogen molecule). This volume, called the gram-molecular volume, is 22,380 c.c. at 0° C. (273° abs.) and 76.0 cm. of mercury pressure (1 atmosphere).

Calling $V$ the volume occupied by a gram-molecule of gas we obtain from the previous equation,

$$R = \frac{pV}{T}.$$

The pressure $p$, per square centimeter of mercury (sp. gr. = 13.59), is equal to 76 cm. × 13.59 = 1033 gms. We obtain, therefore, for the constant $R$,

$$R = \frac{1033 \times 22{,}380}{273} = 84{,}683.$$

To prove the identity of this constant for the osmotic pressure of sucrose one of the experiments of Pfeffer may be selected. A 1 per cent solution of sucrose at 0° C. (273° abs.) gave an osmotic pressure of 49.3 cm. of mercury. The latter corresponds to a pressure per square centimeter of 49.3 × 13.59 = 670 gms. Since the molecular weight of sucrose is 342, the volume ($V$) of a 1 per cent solution containing a gram-molecule would be very closely 34,200 c.c. Substituting these volumes in the equation, we obtain,

$$R = \frac{670 \times 34{,}200}{273} = 83{,}934,$$

which value is in substantial agreement with that derived by the other method.

* Ostwald's " Grundriss " (2nd Ed.), p. 131.

**Application of the Method.** — If we accept now the identity of the laws for gaseous and osmotic pressure, the molecular weight of a sugar can be determined from its osmotic pressure in a manner analogous to that followed by the vapor-density method.

*Example.* — In one of the experiments previously cited Pfeffer found at 15.5° C. (288.5° abs.) for a 1 per cent sucrose solution an osmotic pressure of 52.05 cm. mercury.

If 1 gm. of sucrose occupies 100 c.c. at 52.05 cm. pressure and 15.5° C., then the number of grams which would occupy 22,380 c.c. at 0° C. (273° abs.) and 76 cm. pressure would be:

$$\frac{1 \text{ gm.} \times 22{,}380 \text{ c.c.} \times 288.5^\circ \times 76 \text{ cm.}}{100 \text{ c.c.} \times 273^\circ \times 52.05 \text{ cm.}} = 345.$$

345 the number of grams in the gram-molecular volume is the molecular weight of sucrose. This agrees closely with the actual value 342 calculated from the formula, $C_{11}H_{22}O_{11}$.

It follows from the previous discussion that the sugars of lowest molecular weight will show for equal concentration and temperature the highest osmotic pressure.

**Measurement of Osmotic Pressure by Plasmolysis.** — A second method of applying the principle just described is due to the Dutch botanist de Vries,* who discovered that the plasmolysis, or loosening of the protoplasmic lining of plant cells, offered a simple and reliable means of measuring osmotic pressure. Fig. 154 shows the microscopic appearance of a plant cell in sugar solutions of different concentration. In such a cell the thin layer $p$ of protoplasm (the protoplast) acts as a semipermeable membrane. So long as the osmotic pressure of the cell liquid $l$ exceeds or equals that of the surrounding sugar solution $s$, the protoplast is not affected. When, however, the osmotic pressure of the sugar solution becomes greater than that of the cell liquid there is a diffusion of water outward through the protoplasmic membrane. The latter, in consequence of the loss of a part of the cell water, is loosened from the cell wall and contracts, as shown in the figure.

The application of the method may be understood from the following: de Vries found that the hair roots of the frogbit (*Hydrocharis Morsus-ranæ*) showed no plasmolysis in a 7 per cent, but a very pronounced loosening of the protoplast in a 7.1 per cent, sucrose solution. For these particular root hairs under the conditions of the experiment, plasmolysis was produced by a solution containing 0.208 gm. mol. of sucrose to 1000 gms. of solution (71 gms. ÷ 342, the molecular weight of sucrose).

* Bot. Ztg., **46**, 229, 393.

Suppose that, using these same root hairs, a solution containing 3.7 per cent of glucose just produced plasmolysis. Then 37 (the grams of glucose per 1000 gms. of solution) divided by 0.208 = 178, the molecular weight of glucose, which corresponds to the formula $C_6H_{12}O_6$ (molecular weight = 180).

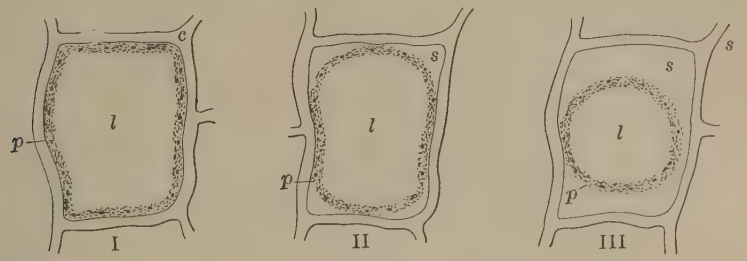



Fig. 154. — Illustrating plasmolysis.
I. Condition of plant cell before plasmolysis; II. Beginning of plasmolysis; III. Advanced stage of plasmolysis.

It was by this means that de Vries,* in 1888, established the molecular weight of raffinose. The following formulæ had been proposed for the constitution of this sugar.

I. $C_{11}H_{22}O_{11} + 3\,H_2O = 396$, molecular weight.
II. $C_{18}H_{32}O_{16} + 5\,H_2O = 594$, molecular weight.
III. $C_{36}H_{64}O_{32} + 10\,H_2O = 1188$, molecular weight.

De Vries found by his method of plasmolysis that, when standardized against a sucrose solution for the same plant cell, 595.7 parts of raffinose were equimolecular with 342 parts of sucrose. This figure agrees with the molecular weight of formula II; the correctness of de Vries's conclusion was afterwards verified by chemical means.

Owing to the variation in composition of cell liquids, it is evident that the particular plant cells chosen for this method of examination must always be standardized before using.

### FREEZING AND BOILING POINTS OF SUGAR SOLUTIONS

On account of the difficulty of preparing a perfect semipermeable membrane and owing to the extreme liability of such membranes to rupture, the determination of molecular weights by direct measurement of osmotic pressure, although most sound in principle, is not generally followed. Use is accordingly made of the measurement of some related constant, such as that of vapor pressure, depression of freezing

* Compt. rend., **106**, 751.

point or elevation of boiling point. The freezing and boiling points of sugar solutions vary in fact according to their vapor pressure, the value of which, it can be shown, is directly proportional to the osmotic pressure.

**Isotonic Solutions.** — In Fig. 155 suppose the closed vessel $V$ to be divided by a semipermeable membrane $M$-$M'$ into two equal compartments, which open into one another above $M$. Suppose, next, equal volumes of sucrose and glucose solutions of the same concentration to be placed in each of the compartments. Then water will diffuse from the sucrose solution $S$, where the osmotic pressure is lower, into the glucose solution $G$, where the osmotic pressure is higher, until at the point of equilibrium the osmotic pressures upon both sides of the membrane are equal. The two sugar solutions are then said to be isotonic and isotonic solutions must have the same vapor pressure. For if the vapor pressures were unequal, water vapor would pass from the solution of higher to that of lower vapor pressure, the concentration of the sugar solutions would thus be changed, and water must again diffuse to the compartment of higher osmotic pressure. There would thus be established a perpetual motion which is contrary to law. Consequently isotonic solutions must have the same vapor pressure.

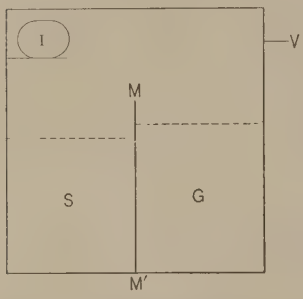


Fig. 155. — Illustrating principle of isotonic sugar solutions.

Suppose next a piece of ice $I$ to be placed in the closed compartment above the partition $M$, and suppose this ice to be of the same temperature as the freezing point of the isotonic sucrose solution $S$. Then the vapor pressure between $I$ and $S$ must be equal, otherwise water vapor would pass between the two and change the freezing point of $S$. But since $S$ and $G$ are both isotonic and have the same vapor pressure, both must also have the same freezing point.

In the same way the two isotonic solutions $S$ and $G$ must have the same boiling point, the vapor tension of the aqueous vapor at the boiling point being the same for both solutions.

The proportionality between changes in vapor pressure and between changes in freezing or boiling point is easily illustrated by means of a diagram. In Fig. 156, let $OW$ be the pressure curve of water for change in temperature and $OI$ the pressure curve of ice, the projection of

*O* at *T* being the freezing point of water. Let *Ss* be the corresponding curve of a 1 per cent sucrose solution and *Gg* of a 1 per cent glucose solution, the projection of the points *s* and *g* at *t* and *t'* being the respective freezing points of the two solutions. For comparatively small areas the lines *gO*, *ss'* and *gg'* may be regarded as straight and *ss'*

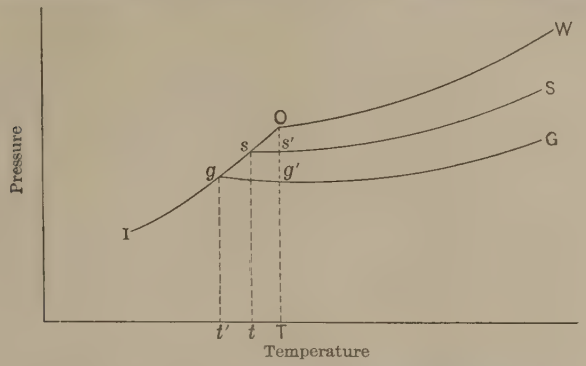



Fig. 156. — Showing relation of vapor pressure of sugar solutions to depression in freezing points.

and *gg'* as parallel. In the $\triangle Ogg'$, $Os' : Og' :: Os : Og$ and so also $Os : Og :: Tt : Tt'$. Therefore the lowerings in vapor pressure (and hence osmotic pressure) *Os'* and *Og'* of the two sugar solutions as compared with the solvent water are directly proportional to the corresponding depressions in freezing point *Tt* and *Tt'*.

**Raoult's Method for Determining Depression of Freezing Point.** — For determining the depression of freezing points by Raoult's * method the apparatus of Beckmann † (Fig. 157) is generally used. This consists of a large tube *A* (2.5 cm. × 21 cm.) provided with a side tube *A'*. The main opening is provided with a stopper through which pass the Beckmann thermometer *D* and a small stirrer, provided with a cork handle *r*. The thermometer has a range of about 6 degrees and the scale is divided into hundredths, the thousandths of a degree being estimated by aid of a magnifying glass. The tube *A* fits through a cork into the larger tube *B*, which serves as an air-jacket, and the whole sets in the cover of a large glass cylinder which is filled with a freezing mixture a few degrees lower than the freezing point of the solution to be examined.

* Compt. rend., **94,** 1517; **101,** 1056; **103,** 1125.
† Z. physik. Chem., **2,** 638.

In making an experiment, using water as the solvent, the freezing bath is set at about $-5°$ C. and the mercury of the Beckmann thermometer adjusted by means of its regulating device *c*, so that the top of the column falls within the proper range of the scale. A weighed quantity of water, sufficient to cover the bulb of the Beckmann thermometer, is placed in *A*, the thermometer and stirrer are inserted and the tube plunged through the small opening *b* into the freezing mixture. When signs of freezing begin to appear, the tube is withdrawn from the freezing mixture, wiped dry and then inserted in the air-jacket *B*. The water and forming ice are now stirred vigorously by *r*; the temperature after reaching a certain minimum begins to increase suddenly with the liberation of latent heat. The mercury soon ceases to rise and the point at which it stops, after tapping to prevent any lag, is taken as the freezing point of the water. The operation is repeated several times and the average of the observations taken as the final value. The same operations are now repeated after introducing through *A′* known weights of the sugar to be examined (1 to 5 gms. per 100 gms. of water), the maximum point to which the mercury rises after overcooling being taken as the freezing point of the solution. The corrected difference between the freezing point of water and that of water + sugar is the depression of freezing point.

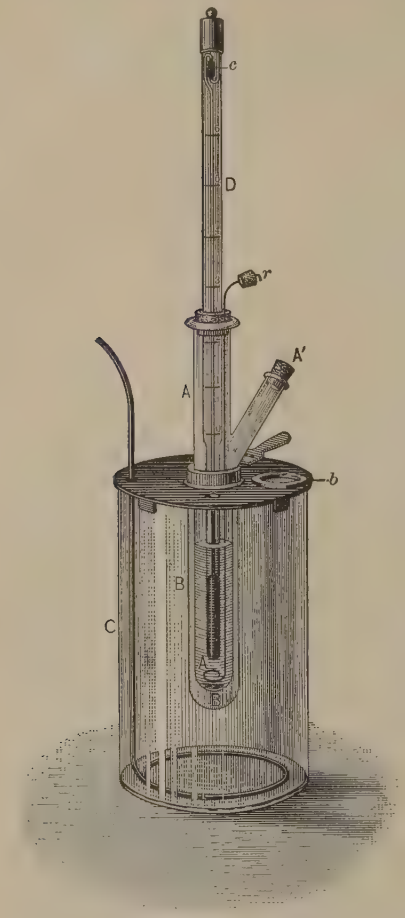



Fig. 157. — Beckmann's apparatus for determining depression of freezing point.

**Molecular Depression of Freezing Point.** — According to what was said under osmotic and vapor pressure, solutions of undissociated substances (non-conducting solutions), which contain the same number of gram-molecules per liter, should show the same depression of freezing point. The depression for 1 gm. mol. of undissociated substance per 1000 gms. of solvent, according to van't Hoff,* is expressed by the formula $\frac{0.002\ T^2}{W}$, in which $T$ is the absolute temperature of melting, and $W$ the latent heat of melting for the solvent. This expression in case of water, whose latent heat of melting is 80 calories and temperature of melting 273° abs., would give $\frac{0.002 \times 273^2}{80} = 1.86$. Loomis, as a matter of fact, in the examination of solutions of some 25 different substances obtained a depression in freezing point for 1 gm. mol. to 1000 gms. of water of almost exactly 1.86° C. The following experiments by Loomis† give the results of 6 tests upon maltose. ($M$, the molecular weight of maltose anhydride $C_{12}H_{22}O_{11} = 342$.)

| Grams maltose to 1000 grams water ($P$). | Gram-molecules of maltose to 1000 grams water $\left(\frac{P}{M}\right)$. | Depression of freezing point ($\Delta$), degrees C. | Molecular depression of freezing point $\left(\Delta \Big/ \frac{P}{M} = \frac{\Delta M}{P}\right)$. |
|---|---|---|---|
| 3.431 | 0.0100 | 0.0193 | 1.86 |
| 6.879 | 0.0201 | 0.0378 | 1.88 |
| 10.350 | 0.0302 | 0.0560 | 1.85 |
| 17.316 | 0.0506 | 0.0946 | 1.87 |
| 35.004 | 0.1023 | 0.1919 | 1.876 |
| 71.548 | 0.2091 | 0.3946 | 1.887 |

**Applications of Freezing-point Method.** — The application of the freezing-point method to the determination of molecular weights may be understood from the following example:

| | Corrected freezing point upon Beckmann scale. |
|---|---|
| 20 gms. of water in the apparatus gave | 4.320° |
| 20 gms. of water + 0.3647 gms. fructose gave | 4.131 |
| Depression of freezing-point ($\Delta$) = | 0.189° C. |

The grams of fructose calculated to 1000 gms. of water would be

$$\frac{0.3647 \times 1000}{20} = 18.235 \text{ gms.} = P.$$

Since $\frac{\Delta M}{P}$ = the constant 1.86, $M = \frac{1.86\ P}{\Delta}$.

* Ostwald's "Grundriss" (2nd Ed.), p. 142.

† Z. physik. Chem., **37**, 407.

Substituting the values obtained for the $\Delta$ and $P$ of fructose we obtain

$$M = \frac{1.86 \times 18.235}{0.189} = 179.5,$$

which agrees closely with the value 180, required by the formula $C_6H_{12}O_6$.

If $w$ is the weight of sugar taken and $W$ the weight of water, the various steps of the calculation are represented by the general equation:

$$M = \frac{w \times 1000 \times 1.86}{W \times \Delta}.$$

The method of determining molecular weight by the depression of freezing point is one that requires considerable care in manipulation, and the inexperienced chemist should thoroughly test the method upon substances of known molecular weight before applying it to the examination of unknown compounds. The method is open to a large number of experimental errors, such as too low a temperature of freezing bath, too high a room temperature, radiation of heat from the observer, faulty thermometer or error in reading, solution of air by the water, careless handling of the instrument, etc. For a thorough discussion of these various points the chemist is referred to the original papers by Raoult, Beckmann, Loomis and others.* Owing to the small value of $\Delta$ any slight error in its determination becomes greatly magnified in the final calculation.

The freezing-point method has been successfully employed by Tollens and Mayer, Brown and Morris, and others in determining the molecular weights of many sugars. The following examples of determinations for nine sugars are selected from a compilation of results by Tollens.†

| Sugar. | Formula. | Molecular weight. | | Authority. |
|---|---|---|---|---|
| | | Calculated. | Found. | |
| Arabinose | $C_5H_{10}O_5$ | 150.08 | 150.3 | Brown and Morris |
| Xylose | $C_5H_{10}O_5$ | 150.08 | 154.1 | Tollens and Mayer |
| Glucose | $C_6H_{12}O_6$ | 180.10 | 179 | Tollens and Mayer |
| Invert sugar | $C_6H_{12}O_6$ | 180.10 | 174.3 | Brown and Morris |
| Galactose | $C_6H_{12}O_6$ | 180.10 | 177 | Brown and Morris |
| Sucrose | $C_{12}H_{22}O_{11}$ | 342.18 | 352 | Raoult |
| Maltose | $C_{12}H_{22}O_{11}$ | 342.18 | 322 | Brown and Morris |
| Lactose | $C_{12}H_{22}O_{11},H_2O$ | 360.19 | 353 | Tollens and Mayer |
| Raffinose | $C_{18}H_{32}O_{16},5H_2O$ | 594.32 | 594 | Tollens and Mayer |

The freezing-point method can be applied to the examination of sugar solutions for other purposes than those of molecular weight de-

* For a complete review and bibliography of the subject see Lippmann's "Chemie der Zuckerarten," 1126. † "Handbuch der Kohlenhydrate," II, p. 26.

termination. Kahlenberg, Davis and Fowler,* for example, have employed it in measuring the speed of inversion of sucrose. Table LXIII, by the above authorities, gives a comparison of the inversion coefficient of sucrose as determined by the polariscope and freezing-point methods. One-half gram molecule of sucrose to 1000 c.c. was inverted at 55.5° C. by $\frac{1}{100}$ gm. mol. of hydrochloric acid.

TABLE LXIII

*Giving Rate of Inversion of Sucrose as Determined by Polariscope and by Depression in Freezing Point*

| Time. | Polariscope reading. | Inversion coefficient $K$ by polariscope. | Depression in freezing point. | Inversion coefficient $K$ by freezing point. |
|---|---|---|---|---|
| Hours. | | | Degrees C. | |
| 0.0 | 22.62 | ..... | 1.175 | ..... |
| 1.0 | 16.58 | 0.0983 | 1.393 | 0.0977 |
| 2.0 | 9.92 | 0.1205 | 1.635 | 0.1217 |
| 2.5 | 7.68 | 0.1208 | 1.705 | 0.1185 |
| 3.0 | 5.94 | 0.1186 | 1.809 | 0.1296 |
| 4.0 | 2.54 | 0.1215 | 1.912 | 0.1263 |
| 4.5 | 1.42 | 0.1198 | 1.954 | 0.1252 |
| 7.0 | −2.40 | 0.1130 | 2.105 | 0.1254 |
| 17.5 | −6.90 | 0.1142 | 2.230 | 0.1028 |
| 26.5 | −7.20 | ..... | 2.247 | ..... |
| | Average..... | 0.1158 | | 0.1147 |

It is seen that the value of the constant $K$, as determined by the Wilhelmy equation $K = \frac{1}{t}\log\frac{a}{a-x}$ (p. 660), is identical by the two methods of measurement.

**Beckmann's Method for Determining Elevation of Boiling Point** — Beckmann's† method of determining molecular weights by the elevation of boiling point is the same in principle as that by depression of freezing point. A gram-molecule solution of an undissociated substance should show according to van't Hoff's formula $\frac{.002\,T^2}{W}$ (in which $T = 373$ degrees, the absolute boiling point of water and $W = 536$ cals., the latent heat of evaporation), an elevation in boiling point of $\frac{.002 \times 373^2}{536} = 0.519° = \Delta$.

Beckmann‡ found in one experiment an elevation in boiling point

* J. Am. Chem. Soc., **21**, 1.

† Z. physik. Chem., **3**, 603; **4**, 532; **5**, 76; **6**, 437; **8**, 223.

‡ Ibid., **6**, 459.

of 0.315° C. for a solution containing 216.8 gms. of sucrose to 1000 gms. of water, or $\frac{216.8}{342} = 0.634$ gm. mols. The elevation in boiling point for a 1 gm. mol. solution would then be $\frac{0.315}{0.634} = 0.497°$ C., which is slightly lower than the value calculated by van't Hoff's formula.

The general formula for calculating molecular weights from the elevation in boiling point ($\Delta$) is similar to the formula for the freezing point method (p. 330) and is

$$M = \frac{w \times 1000 \times 0.52}{W \times \Delta}$$

The boiling-point method, upon the whole, is open to more sources of error than the freezing-point method and has proved much less satisfactory as a means of establishing the molecular weights of sugars.

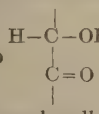
# CHAPTER XIII

## QUALITATIVE METHODS FOR THE IDENTIFICATION OF SUGARS

PROBABLY no other class of organic compounds gives such a variety of reactions, or forms so large a number of chemical derivatives as the sugars. Owing to the great extent of the field it will be possible to describe only a few of the more general tests and reactions.

In describing the various chemical tests, the sugars will be classified for convenience under two general groups: I. The reducing sugars. II. The non-reducing sugars. The reducing sugars are distinguished by the fact that they cause a marked precipitation of cuprous oxide when warmed with Fehling's alkaline copper solution, whereas the non-reducing sugars do not exhibit this property, or only to a very slight extent after prolonged boiling. The reducing sugars constitute by far the larger group; of the some one hundred known natural or synthetic sugars, about ninety are reducing and only about ten non-reducing.

### Reactions of the Reducing Sugars

The characteristic chemical properties of the reducing sugars are due for the most part to the occurrence of a common carbonyl-alcohol group  . The reducing sugars, as aldoses or ketoses, give in fact nearly all the reactions peculiar to aldehydes and ketones. The chemist must, therefore, first of all, guard against deciding as to the presence of a sugar from a reaction which would also be given by formaldehyde, acetaldehyde or acetone. A number of confirmatory tests must usually be applied, before it can be stated definitely whether a sugar is or is not present.

The qualitative reactions for reducing sugars are divided for convenience into I. General tests; II. Special tests; III. Individual tests. After it has been determined from general tests that a sugar is present, special tests must be applied in order to determine what classes or groups of sugars are present, whether hexoses or pentoses, aldoses or ketoses, monosaccharides or disaccharides. After the class or group of sugars has been ascertained, individual tests must be applied in order

to determine what particular sugars are present. Only the general and special tests are taken up in the present chapter. The individual tests are given under the description of the different sugars in Part II.

### General Tests for Reducing Sugars

Among the general tests which are sometimes given for sugars may be mentioned the familiar property which all carbohydrates have of giving off a characteristic sweetish odor upon heating over a flame in a closed tube. This odor, which is usually designated as caramel-like, is given off, however, by many polyatomic alcohols and acids (as by tartaric acid) so that the test is not characteristic of sugars alone. Among the decomposition products obtained by heating sugars in a closed tube may be mentioned (besides water and the gaseous products carbon dioxide and carbon monoxide) formic acid, acetic acid, acetone, furfural and various products of an aldehyde nature. It is to the furfural and aldehyde products that the characteristic odor of burnt sugar is largely due.

The general tests for reducing sugars may be divided for convenience into four general groups of reactions.

I. Reducing reactions with alkaline solutions of metallic salts.
II. Color reactions with alkalies, acids and phenols.
III. Hydrazone and osazone reactions with phenylhydrazine and its substituted derivatives.
IV. Miscellaneous reactions.

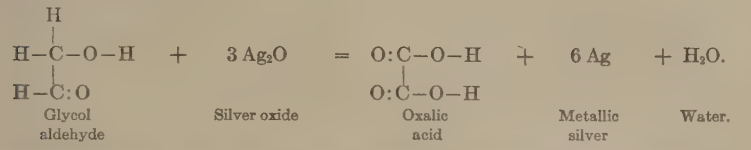
### I. Reducing Reactions of Sugars with Alkaline Solutions of Metallic Salts

The simple sugars and certain of the disaccharides, as maltose and lactose, have the property of reducing alkaline solutions of many metallic salts, such as those of copper, silver, mercury and bismuth. This reaction, which is common to most aldehydes, is due to the withdrawal of oxygen from the metallic base, the latter being precipitated either as a suboxide or in the metallic form. The aldehyde group of the sugar molecule is oxidized by the oxygen withdrawn from the metallic base to the acid carboxyl group, as indicated by the following general equation:

$$\underset{\text{Aldehyde}}{H-\overset{|}{C}:O} + \underset{\text{Copper Oxide}}{2\,CuO} = \underset{\text{Acid}}{H-O-\overset{|}{C}:O} + \underset{\text{Copper Suboxide.}}{Cu_2O.}$$

The above, however, marks only the beginning of the reaction, for, upon heating, the oxidation of the sugar molecule usually proceeds with the

conversion of alcohol into carboxyl groups as in the following reaction for glycol aldehyde:




This oxidation in the case of the higher monosaccharides is usually attended by a breaking down of the carbon chain as by the oxidation of glucose in ammoniacal silver solution:

$$C_6H_{12}O_6 + 9\ Ag_2O = 3(COOH)_2 + 18\ Ag + 3\ H_2O.$$

The reaction between sugars and alkaline salts of metals, as ordinarily carried out, gives rise to a number of monobasic and dibasic acids (formic, oxalic, etc.) in varying proportions according to the conditions of the experiment. It is not possible, therefore, to express the reaction by chemical equations except in a very general way.

The most common of the alkaline salt solutions employed in testing sugars are those of copper. The sulphate and acetate of copper are the salts most generally used and sugar literature is filled with descriptions of modifications for making the test. Only a few of these will be described.

**Fehling's Copper Solution.** — This is the most common chemical reagent employed in testing sugars. As ordinarily prepared the reagent consists of two solutions: solution *A* containing 34.64 gms. crystallized copper sulphate to 500 c.c. and solution *B* containing 173 gms. Rochelle salts and 51.6 gms. sodium hydroxide to 500 c.c. The solutions are the same as those used in quantitative analysis and are to be kept separate until just before using. By mixing 5 c.c. each of solutions *A* and *B* in a test tube, adding a few c.c. of the solution to be examined and heating to boiling for 2 minutes, a brick-colored precipitate of cuprous oxide, $Cu_2O$, will form, if reducing sugars are present, the intensity of coloration and amount of precipitate being proportional to the amount of sugar present. The test is sensitive to about 0.01 mg. of glucose to 1 c.c.

*Products Obtained by Heating Reducing Sugars with Fehling's Solutions.* — The chemical reactions which take place in the oxidation of sugars by means of Fehling's solution are exceedingly complex. Nef,* who has made the most complete studies in this field, found that in case of l-arabinose, the oxidation proceeds along three separate lines.

* Ann., **357**, 214–312.

I. From 10 to 25 per cent of sugar are oxidized to form pentonic acids.

$$C_5H_{10}O_5 + O = C_5H_{10}O_6.$$

II. From 35 to 45 per cent of sugar are oxidized to form formic and trioxybutyric acids.

$$C_5H_{10}O_5 + 2\,O = HCOOH + C_4H_8O_5.$$

III. From 30 to 38 per cent of sugar are oxidized to form formic and glycollic acids.

$$C_5H_{10}O_5 + 3\,O = HCOOH + 2\,C_2H_4O_3.$$

In case of the hexose sugars, d-glucose, d-mannose and d-fructose, Nef obtained analogous reactions with formation of carbonic, formic, glycollic, glyceric, trioxybutyric and hexonic acids. The amount of the different acids was found to vary according to the amount of alkali present.

In testing solutions containing much foreign organic matter such as urine, the reaction with Fehling's solution may be interfered with. Uric acid, creatine, creatinine, albumin, peptones and other substances may either check the precipitation of cuprous oxide, when reducing sugars are present, or in some cases cause a precipitate of copper in the complete absence of sugars. Solutions containing xanthine bases, such as low-grade molasses, distillery waste, etc., when heated with Fehling's solution may precipitate greenish-yellow copper compounds, which may be mistaken for cuprous oxide. In all such cases the impure solution should be clarified with a little normal acetate of lead and filtered; any excess of lead is removed from the filtrate with sodium carbonate and the clear solution tested with Fehling's reagent in the usual way. Filtering the impure solution through animal charcoal is also of advantage when foreign coloring matter masks the reaction.

**Barfoed's Copper Solution.** — Instead of the sulphate, solutions of other copper salts have been employed in testing for sugars. Barfoed * has prepared a solution containing one part crystallized neutral copper acetate in 15 parts of water; 5 c.c. of 38 per cent acetic acid are added to 200 c.c. of the copper-acetate solution before use. On boiling the solution a basic acetate of copper is formed, the liberated cupric oxide being reduced in presence of monosaccharides. Barfoed's reagent is not reduced to any great extent by the disaccharides, lactose and maltose, and is, therefore, of value in distinguishing these sugars from monosaccharides.

* Z. analyt. Chem., **12**, 27.

**Soldaini's Copper Solution.** — Carbonate of copper solution has also been used in testing for sugars. Soldaini * has prepared a solution containing 15 gms. precipitated copper carbonate, $CuCO_3$, and 416 gms. potassium bicarbonate, $KHCO_3$, dissolved to 1400 c.c. Instead of starting with copper carbonate, copper sulphate may be used; a solution of the latter is added to the $KHCO_3$ solution, the precipitate of $CuCO_3$ first formed being dissolved in the excess of bicarbonate. A solution containing 3.464 gms. copper sulphate and 297 gms. potassium bicarbonate to 1000 c.c. is especially adapted for detecting small amounts of reducing sugars.

Among other copper solutions recommended for testing sugars may be mentioned copper ammonium tartrate and ammoniacal copper sulphate or acetate. None of these preparations has been found, however, to equal Fehling's reagent for general usefulness in practical sugar analysis.

**Tollens's Silver Solution.** — The most sensitive of metallic-salt solutions for detecting sugars is ammoniacal silver solution, first employed by Tollens † and hence usually known as Tollens's reagent. This is prepared by dissolving one part silver nitrate in 10 parts of water; a second solution is then made containing one part sodium hydroxide in 10 parts of water. Before making the test equal parts of the two solutions are mixed and then ammonia added drop by drop until the precipitate of silver oxide is completely dissolved. A solution containing one part of glucose in 1000 parts of water will cause a strong reduction of Tollens's reagent in the cold, a mirror of silver being deposited within 15 minutes. A solution containing one part glucose to 100,000 parts of water will also produce a perceptible reduction in the cold, but the solution must stand one to two days. The reduction takes place more rapidly upon warming, but warming or heating the solution is to be avoided owing to the danger of forming explosive silver compounds. For the latter reason the reagent should be prepared only just before using. Tests should be carried out in the dark and solutions containing the reagent should not be kept for any length of time.

Tollens's silver reagent is also reduced by all aldehyde substances; it is affected not only by the sugars which reduce Fehling's solution but also by sucrose, raffinose and all other soluble carbohydrates. Even the alcohol derivatives of the sugars produce reduction, glycerol, for example, causing the formation of a silver mirror. The readiness with which ammoniacal silver solution is reduced by soluble organic

* Z. Ver. Deut. Zuckerind., **39,** 933; **40,** 792.

† Ber., **15,** 1635; **16,** 921.

non-sugars has proved a serious objection against the use of this reagent in ordinary analytical work.

**Knapp's Mercury Solution.**—A third reagent which has been used for testing sugars is Knapp's* alkaline mercuric-cyanide solution. The latter contains 10 gms. of mercuric cyanide dissolved in 100-c.c. sodium hydroxide solution of 1.145 specific gravity. Similar alkaline solutions have been prepared by Sachsse † from mercuric iodide and by Bauer ‡ from mercuric chloride. These solutions are reduced upon warming with sugar solutions giving grayish deposits of metallic mercury. The mercury solutions have the same objection, however, as those of silver in being reduced by different organic non-sugars, such as creatine, creatinine and glycerol and even under certain conditions by alcohol. Alkaline solutions of mercury salts are, therefore, of but little value in detecting sugar in urine and other liquids rich in organic non-sugars.

**Nylander's Bismuth Solution.** — A fourth reagent, which has been used considerably for detecting reducing sugars in urine, is an alkaline solution of bismuth sub-nitrate, known as Nylander's § (or Almen's) reagent. This solution as prepared by Nylander is made by dissolving 2 gms. of bismuth sub-nitrate and 4 gms. of Rochelle salts in 100 gms. of 8 per cent sodium hydroxide solution. After standing for a few days the solution is filtered through glass wool and the clear filtrate preserved in a stoppered bottle. The solution will keep indefinitely. When Nylander's reagent is heated with a solution containing reducing sugars a precipitate of dark metallic bismuth is produced. Heating with $\frac{1}{10}$ its volume of 0.01 per cent glucose solution will cause a perceptible darkening. In testing urine 1 c.c. of the reagent and 10 c.c. of urine are heated in a test tube 2 to 5 minutes over the flame; after standing for 5 minutes the solution is examined for the appearance of a dark-colored sediment.

Nylander's reagent, however, is open to the same objections noted for the alkaline silver and mercury solutions. The presence of albumin, nuclein, glucuronic acid and other organic non-sugars in urine will also cause a precipitation of bismuth, even when glucose is completely absent. While the failure of a precipitate with Nylander's reagent may indicate the absence of reducing sugars, the occurrence of a precipitate may be said to indicate the presence of sugar only when reducing non-sugars are proved to be absent.

* Z. analyt. Chem., **9,** 395.
† Z. Ver. Deut. Zuckerind., **26,** 872.
‡ Landw. Vers.-Stat., **36,** 304.
§ Z. physiol. Chem., **8,** 175.

**Miscellaneous Solutions.** — Of other alkaline solutions of metallic salts proposed for sugar testing may be mentioned alkaline nickel sulphate and tartaric acid which gives a dark-red precipitate of nickel suboxide in presence of reducing sugars, and alkaline ferric chloride and sodium tartrate which gives a brown-colored precipitate on heating with reducing sugars. None of these reagents, however, or any of the other alkaline solutions of metallic salts previously mentioned, has been found to equal Fehling's copper reagent for all-around usefulness and reliability.

## II. COLOR REACTIONS OF SUGARS WITH ALKALIES, ACIDS AND PHENOLS

As a second general reaction of reducing sugars may be mentioned certain color effects which nearly all soluble carbohydrates give when brought into contact with different reagents. The reagents employed may be divided into three groups:

I. Alkalies.
II. Concentrated mineral acids.
III. Phenols.

**Color Reactions of Sugars with Alkalies.** — All reducing sugars have the property of coloring solutions of the alkalies and alkaline earths yellow, the application of heat turning the color a dark brown. This reaction is common to all aldehydes. The exact nature of the coloring matter formed by the action of alkalies upon sugars in solution is not understood. Considerable oxygen is absorbed from the air during the reaction and a variety of products of an acid nature are among the substances formed.

*Products Obtained by Heating Reducing Sugars with Alkali.* — Lactic acid is produced in considerable amount by the action of alkalies upon many reducing sugars such as xylose, arabinose, glucose and fructose. The presence of calcium lactate in certain sugar-cane molasses is explained by the action of an excess of lime during clarification upon the reducing sugars of the juice. Formic, acetic and oxalic acids have also been found among the products resulting from the action of alkalies upon sugars in solution. Certain phenol bodies such as pyrocatechin and protocatechuic acid have also been detected among the oxidation products of sugars resulting from treatment with alkalies.

Nef* has studied the action of $\frac{1}{8}$ normal sodium hydroxide upon different sugars and obtained in case of d-glucose, d-mannose, and d-fruc-

* Ann., **376**, 1–119.

tose a yield of from 40 to 45 per cent d,l-lactic acid, from 10 to 15 per cent d,l-1-hydroxybutyrolactone, about 25 per cent of saccharin, metasaccharin and isosaccharin and a small quantity of tarry decomposition products.

The action of dilute alkalies in causing transformations of sugars into one another by molecular rearrangement is referred to elsewhere.

**Color Reactions of Sugars with Mineral Acids.** — Treatment of solutions of sugars and carbohydrates with concentrated mineral acids gives rise to a number of decomposition products, the color of which frequently throws some light upon the nature of the sugars present. The acids most commonly used for this purpose are sulphuric and hydrochloric. The character of the color generated will depend partly upon the kind of sugar, partly upon the strength of acid used and partly upon the temperature of the reaction.

*Products Obtained by Heating Sugars with Acids.* — The darkening produced in all sugar solutions upon warming with concentrated sulphuric or hydrochloric acid is due largely to the formation of insoluble so-called "humus" substances of relatively high carbon content (C = 62 to 67 per cent and H = 3.5 to 4.5 per cent), the percentage of carbon and depth of color increasing with the strength of acid used. Attempts have been made to classify the humus substances formed by the action of acid upon sugars into ulmin and humin and ulmic and humic acids, to which various formulæ have been assigned by different authorities. The constitution of the humus substances has not been definitely settled, however, and until considerable more work has been done the formulæ of these must remain more or less a matter of conjecture.

In addition to the insoluble humus substances a number of soluble and volatile products are formed by the action of sulphuric and hydrochloric acids upon sugars. Among such products may be mentioned formic acid, levulinic acid, furfural, methylfurfural, oxymethylfurfural and a number of dextrin-like condensation or reversion products of high specific rotation. The nature and amount of these various products depend largely upon the kind of sugar, and a number of methods of group distinction are based upon the separation of characteristic decomposition products. Further reference will be made to these under the special reactions.

The ketoses are much more easily decomposed by strong mineral acids than the aldoses and their solutions give rise to color reactions with corresponding greater facility. This offers one means of distinguishing between a ketose and aldose or of detecting a ketose sugar in presence of an aldose. If a cold sugar solution be treated in a test

tube with a few cubic centimeters of concentrated sulphuric acid, allowing the latter to flow down the walls of the tube to the bottom without shaking, a brown ring will quickly form at the junction of the acid and sugar solution if fructose, sucrose or a sugar containing the ketone group is present; with glucose, lactose, maltose and the aldoses in general no such coloration will develop.

**Color Reactions of Sugars with Phenols.** — The most distinctive color reactions of the sugars are those obtained by treatment with different phenols in presence of concentrated hydrochloric or sulphuric acid. The development of a color in this case is due to the formation of condensation products between the phenol derivatives and the decomposition products obtained from the sugar (humus substances, furfural, aldehydes, etc.). $\alpha$-Naphthol, thymol, resorcin, orcin, naphthoresorcin and phloroglucin are among the more important phenol derivatives used for making color reaction with sugars.

The color reactions with the phenols are performed in various ways. The test with $\alpha$-naphthol, for example, which is perhaps used more frequently than any of the others, is made as follows: 1 to 2 cubic centimèters of the sugar solution are treated in a test tube with 1 to 2 drops of a 10 to 20 per cent alcoholic solution of $\alpha$-naphthol. A few cubic centimeters of concentrated sulphuric acid (must be free from nitric acid) are then carefully added so as to flow down the walls of the tube to the bottom. If sugars containing a ketone group are present a violet ring will form instantly at the junction of the two liquids; in presence of aldoses a gentle warming of the test tube is usually necessary in order to bring out the full intensity of color. The $\alpha$-naphthol test, which is of extreme delicacy, is frequently employed in sugar houses and refineries in testing the condensation water from the vacuum pan for presence of sucrose lost by entrainment.

If the reaction described for $\alpha$-naphthol is carried out with thymol, menthol, resorcin and other phenols similar colorations are produced, the tints varying from cherry red to deep purple.

The tests with phenols and hydrochloric acid are usually made by warming a few cubic centimeters of the sugar solution with a solution of the phenol (resorcin, orcin, phloroglucin, etc.) in concentrated hydrochloric acid. The colorations thus obtained are usually very brilliant, varying in tint from a bright red to a bluish violet. The colors formed are not permanent, however; they rapidly darken and the clear-colored solution soon becomes turbid with the precipitation of a dark-colored condensation product.

USE OF THE SPECTROSCOPE IN STUDYING COLOR REACTIONS FOR SUGARS

The spectroscope has been used with great success by Tollens and his coworkers in studying the colors obtained by treating sugars with different reagents. The appearance of characteristic absorption bands in different parts of the spectrum, when the colored solution is viewed through the spectroscope against white light, is peculiar of many sugars.

**Description of Direct-vision Spectroscope.** — A simple type of spectroscope for studying absorption spectra is the direct vision in-

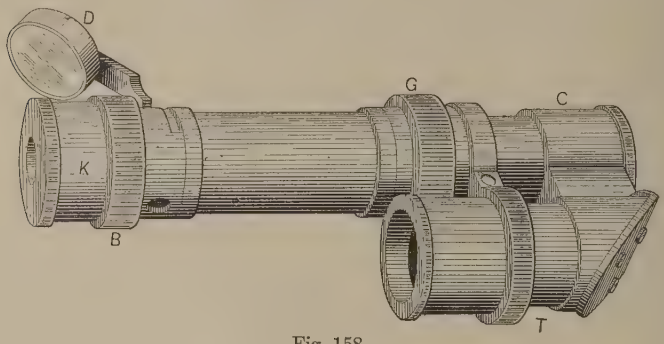



Fig. 158.

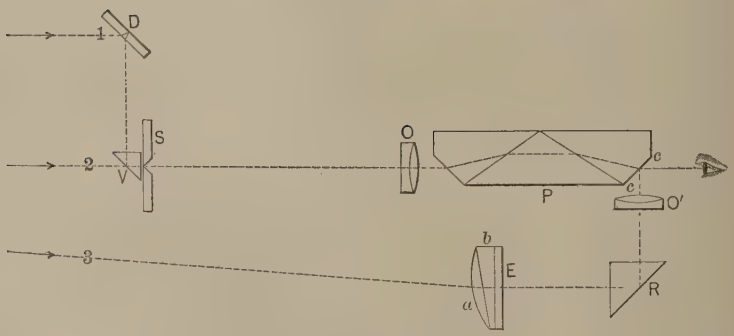



Fig. 159.
Showing outer and inner construction of a direct-vision spectroscope.

strument illustrated in Fig. 158, the interior construction of which is shown in Fig. 159.

The essential parts of the apparatus consist of a telescopic tube containing an Amici prism *P* and an achromatic objective *O*. At one end of the tube, protected by the screw cap *K*, a diaphragm is situated con-

taining a narrow slit *S*, the width of which can be adjusted by turning the milled ring *B*. The upper half of the slit is covered with a small prism *V*; a mirror *D*, which can be rotated through a small angle about the axis of the tube, is also attached to the slit end of the instrument.

At the prism end of the spectroscope there is fixed a small lateral tube *T* containing a graduated scale *E*. The latter is attached to a small prism *b* to which is fixed a converging lens *a*. At *R* is a right angle prism, from the hypotenuse surface of which the image of the scale *E* is reflected through the achromatic objective *O'* upon the cut surface *cc* of the Amici prism.

If the slit end of the spectroscope be pointed towards a sodium flame the rays of light will pass into the spectroscope along the paths 1, 2 and 3. The telescope is first focused by turning the milled ring *G* until a sharply defined image of the lower uncovered half of the slit is obtained by the light passing along 2 upon the surface *cc*. The image of the scale *E* is reflected at the same time, by the light passing along 3, also upon *cc*. The position of the sodium line is noted upon the graduated scale, the latter being in this way standardized. If the spectroscope be now directed towards the sky a continuous spectrum is obtained upon the surface *cc*; the mirror *D* is next turned until the light passing along 1 is reflected through an opening in the cap *K* upon the small prism *V* and thence through the upper half of the slit *S*; in this way a continuous spectrum is obtained upon *cc* the width of which is equal to the total length of the slit *S*.

If the slit has been sufficiently reduced in width, the spectrum of sunlight is seen to be crossed by a number of dark lines, the so-called Fraunhofer lines, which are due to the absorption of certain rays of light from the incandescent mass of the sun by the vaporized elements of the solar atmosphere. A dark line (the *D* line of Fraunhofer's scale), for example, corresponds to the position of the bright-yellow line obtained with the sodium flame and so of the other elements. The position and wave-length of the more important Fraunhofer lines is shown in Fig. 165 (p. 384); their presence is very helpful in defining the position of absorption spectra.

For studying absorption spectra the spectroscope is mounted upon a stand as shown in Fig. 160, a screen *L* being attached to the tube to shade the eye of the observer. The solution to be examined is placed in a small cell *T*, before the front opening in the screw cap and viewed against white light. The rays of light absorbed by the solution will cause characteristic dark-colored bands to appear upon that part of the spectrum corresponding to the lower half of the slit. The part of the

spectrum corresponding to the half of the slit covered by the prism *V* meanwhile remains continuous and together with the scale, or Fraunhofer lines, serves for the exact location of the absorption bands.

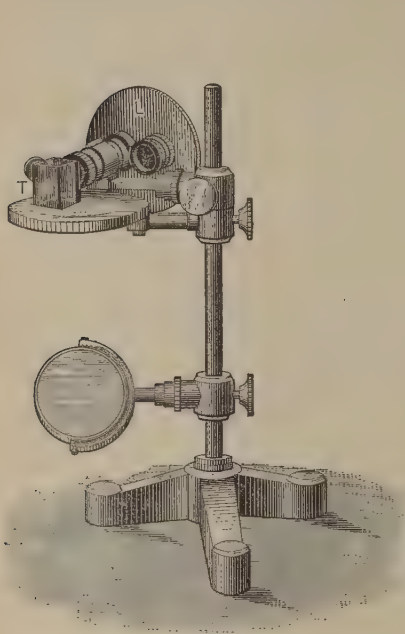



Fig. 160.

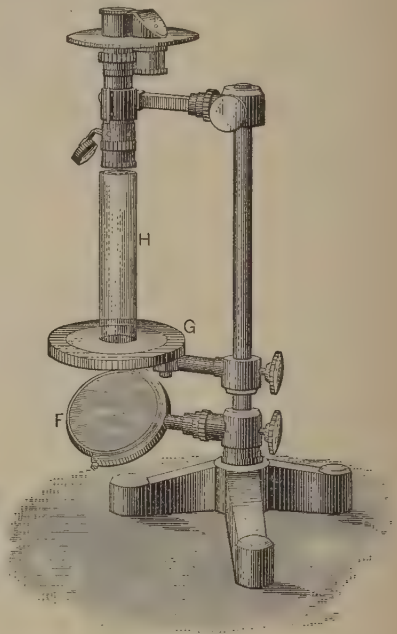



Fig. 161.

Methods of mounting apparatus for study of absorption spectra.

Solutions which are only weakly absorptive are best examined through a large tube *H*, in the manner shown in Fig. 161. The spectroscope is turned and clamped in a vertical position and the light reflected upward from the mirror *F* through the glass bottom of the support *G*.

**Tollens's Method of Studying Absorption Spectra.**—In preparing color tests of sugar solutions for spectroscopic examination it is important that the color remain permanently in solution and that no turbidity develop which would obscure the visible parts of the spectrum. This is sometimes accomplished by carrying out the reaction in presence of alcohol or some other solvent to hold the color compound in solution. A better way is by use of Tollens's* deposit method ("Absatzmethode"). In this method the deposit of insoluble condensation prod-

* Ber., **29**, 1202.

ucts obtained by treating the sugar solution with hydrochloric acid and the phenol (orcin, phloroglucin, naphthoresorcin, etc.) is filtered off, washed several times with water and then dissolved in alcohol. Bright-colored solutions are thus obtained which can be brought by dilution with alcohol to the degree of intensity suitable for spectroscopic examination. Descriptions of characteristic absorption spectra will be given under the reactions for groups and individual sugars.

Of less importance than the color reactions with phenols are the color tests obtained by treating sugars with aromatic amines (aniline, xylidine, diphenylamine, etc.) in presence of concentrated hydrochloric acid. The colors in this instance are due to a combination between the aromatic amine and the furfural, methylfurfural, and oxymethylfurfural derived from the decomposition of the sugar.

### III. HYDRAZONE AND OSAZONE REACTIONS OF REDUCING SUGARS WITH PHENYLHYDRAZINE AND ITS SUBSTITUTED DERIVATIVES

In many respects the most important of the qualitative tests for sugars are those obtained with phenylhydrazine and its substituted derivatives. Phenylhydrazine was introduced as a reagent in sugar chemistry by Emil Fischer* in 1884; it has been of immense service not only as a means of separation and identification but also in first opening a way to a thorough understanding of the molecular constitution of sugars.

**Hydrazone Reaction.** — The reaction with phenylhydrazine is limited to such sugars as contain a free carbonyl group and proceeds in two phases with production of two entirely different classes of compounds. The first phase of the reaction is common to all aldehydes and ketones, the O of the carbonyl group combining with $H_2$ of the amino group in the phenylhydrazine with formation of a group of compounds called hydrazones. With formaldehyde, for example, the reaction proceeds as follows:

$$\underset{\text{Formaldehyde}}{H_2C:O} + \underset{\text{Phenylhydrazine}}{H_2N-NHC_6H_5} = \underset{\text{Formaldehyde-phenylhydrazone}}{H_2C:N-NHC_6H_5} + \underset{\text{Water}}{H_2O}$$

With the carbonyl group of a sugar the reaction would be for a diose:

$$\underset{\text{Diose}}{\begin{matrix}CH_2OH\\|\\HC:O\end{matrix}} + \underset{\text{Phenylhydrazine}}{H_2N-NHC_6H_5} = \underset{\text{Diose-phenylhydrazone}}{\begin{matrix}CH_2OH\\|\\HC:N-NHC_6H_5\end{matrix}} + \underset{\text{Water}}{H_2O}$$

* Ber., **17**, 579.

The hydrazone reaction is carried out by treating the sugar solution in the cold with a solution containing one volume of phenylhydrazine, one volume of 50 per cent acetic acid, and three volumes of water. A little more of the phenylhydrazine is used in making the test than the theoretical quantity corresponding to the supposed amount of sugar present. In place of the above solution the crystalline chloride of phenylhydrazine may be used to advantage, a few grams of sodium acetate being also added to promote the reaction. After the above treatment the hydrazones of the sugars will separate sooner or later as well-defined crystalline compounds, the length of time for separation depending upon the solubility of the hydrazones formed. The phenylhydrazone of mannose, for example, being very insoluble, will separate almost immediately; those of the methylpentoses, fucose, rhamnose and rhodeose also deposit readily; the phenylhydrazone of glucose, on the other hand, which is quite soluble in water, may require one or two days for its precipitation. By filtering off the hydrazones as they are formed a separation of sugars in mixtures may often be accomplished.

After separation of the hydrazones the latter are filtered off and recrystallized either from water or, in case of difficultly soluble hydrazones, from alcohol or pyridine.

*Use of Substituted Derivatives of Phenylhydrazine.* — In place of phenylhydrazine any of its substituted derivatives may be used for the purpose of precipitating sugars. The substituted phenylhydrazines yield in many cases characteristic hydrazones with sugars and their use in sugar chemistry in recent years has been of the greatest service. Of the various substituted phenylhydrazines the following are among the most important.

1. Methylphenylhydrazine $H_2N-N\begin{matrix}\nearrow CH_3\\ \searrow C_6H_5\end{matrix}$
2. Ethylphenylhydrazine $H_2N-N\begin{matrix}\nearrow C_2H_5\\ \searrow C_6H_5\end{matrix}$
3. Amylphenylhydrazine $H_2N-N\begin{matrix}\nearrow C_5H_{11}\\ \searrow C_6H_5\end{matrix}$
4. Allylphenylhydrazine $H_2N-N\begin{matrix}\nearrow C_3H_5\\ \searrow C_6H_5\end{matrix}$
5. Diphenylhydrazine $H_2N-N\begin{matrix}\nearrow C_6H_5\\ \searrow C_6H_5\end{matrix}$
6. Benzylphenylhydrazine $H_2N-N\begin{matrix}\nearrow C_7H_7\\ \searrow C_6H_5\end{matrix}$

7. Parabromophenylhydrazine $H_2N-N\begin{matrix}H\\ \diagdown C_6H_4Br\end{matrix}$

8. Paranitrophenylhydrazine $H_2N-N\begin{matrix}H\\ \diagdown C_6H_4NO_2\end{matrix}$

Other hydrazines than those of the phenyl group are also employed as, for example,

9. Naphthylhydrazine $H_2N-N\begin{matrix}H\\ \diagdown C_{10}H_7\end{matrix}$

The reactions with the substituted hydrazines are usually best carried out in alcoholic solution, the hydrazones formed being for the most part much less soluble than those of ordinary phenylhydrazine.

In the examination of the hydrazones obtained from sugar solutions a melting point of the product is taken before and after recrystallization. If the melting point remains unchanged the hydrazone is pure. Should a difference in the temperature of melting be obtained the hydrazone should be recrystallized until successive determinations show no change in melting point. A table of melting points will then usually identify the hydrazone of the sugar. (See Table 24, Appendix.)

*Separation of Sugars from Hydrazones.* — When a sufficient quantity of hydrazone is available it is always well to decompose the compound and make a direct examination of the separated sugar. For the separation of sugars from their hydrazones two processes are available: First, by means of concentrated hydrochloric acid as originally used by Fischer. Second, by means of benzaldehyde and formaldehyde as recommended by Herzfeld* and by Ruff.†

When the hydrazone of a sugar is treated with concentrated hydrochloric acid the chloride of the hydrazine and free sugar are formed: —

$$\underset{\text{Hexose-phenylhydrazone}}{C_6H_{12}O_5N-NHC_6H_5} + HCl + H_2O = \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\text{Phenylhydrazine chloride}}{HCl\,H_2N-NHC_6H_5}$$

The phenylhydrazine chloride is almost insoluble in concentrated hydrochloric acid and is removed by filtration. The filtrate is neutralized with lead carbonate; the lead chloride is filtered off and the filtrate evaporated to a syrup. The latter is shaken with 95 per cent alcohol, any remaining lead chloride filtered off and the alcoholic filtrate evaporated to a sirup which is set aside for the sugar to crystallize.

* Ber., **28,** 442.
† Ber., **32,** 3234.

The separation of sugars from their hydrazones by means of aldehydes is much simpler than by use of hydrochloric acid and this is the process most generally used at present. For this purpose benzaldehyde is usually employed for the hydrazones of phenylhydrazine and formaldehyde for the hydrazones of the substituted hydrazines. The reaction between the aldehyde and hydrazone is a simple one, the aldehyde displacing the sugar with formation of aldehyde hydrazone.

$$\underset{\text{Hexose-phenylhydrazone}}{C_6H_{12}O_5N-NHC_6H_5} + \underset{\text{Benzaldehyde}}{C_6H_5CHO} = \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\text{Benzaldehyde-phenylhydrazone}}{C_6H_5CHN-NHC_6H_5}$$

$$\underset{\text{Hexose-diphenylhydrazone}}{C_6H_{12}O_5N-N(C_6H_5)_2} + \underset{\text{Formaldehyde}}{CH_2O} = \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\text{Formaldehyde-diphenylhydrazone}}{CH_2N-N(C_6H_5)_2}$$

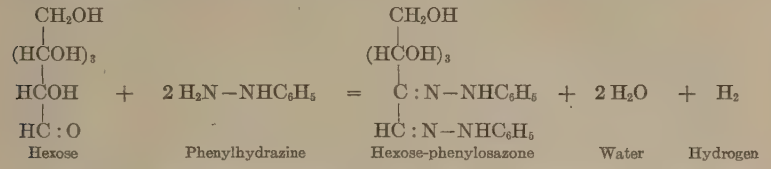
The reaction is best carried out by treating a solution of the hydrazone in 50 per cent alcohol in a flask with an amount of the aldehyde slightly in excess of the theoretical quantity necessary to effect decomposition. The flask is then attached to a reflux condenser and the solution gently boiled for an hour. After cooling, the solution is filtered from the aldehyde hydrazone, the filtrate shaken out several times with ether in a separatory funnel, the sugar solution, after decolorizing with animal charcoal, evaporated to a sirup and set aside for crystallization. Should crystallization not take place immediately, the process may be promoted by priming the sirup with a minute crystal of the sugar suspected to be present. After crystallization the sugar crystals are filtered off, washed with alcohol and ether (using suction) and dried between filter paper in a desiccator over concentrated sulphuric acid. The identity of the sugar thus obtained is then established by determination of its specific rotation.

If the filtrate obtained from filtration of a hydrazone be shaken out with ether to remove excess of hydrazine, the solution can be treated a second time with a different hydrazine. In this manner a qualitative separation of several mixed sugars may be accomplished.

**Osazone Reaction.** — While the hydrazone reaction is of preeminent value in the isolation of sugars, the osazone test with phenylhydrazine is usually of more qualitative significance owing to the greater insolubility of the osazones in water and the consequent greater rapidity and ease of their separation as compared with hydrazones.

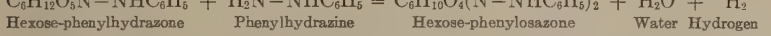
If a solution of a reducing sugar be treated with an excess of phenylhydrazine and then warmed, two molecules of phenylhydrazine unite with the sugar molecule forming an osazone. The aldehyde or ketone

group of the sugar and the *adjacent* alcohol group are the ones which always participate in this reaction.




The free hydrogen liberated in the above reaction acts upon a part of the excess of phenylhydrazine reducing this to aniline with liberation of ammonia.

Since the first stage in the reaction with phenylhydrazine is the formation of a hydrazone, it follows that all phenylhydrazones when treated with phenylhydrazine in excess are changed to the corresponding osazones.




In conducting the reaction for osazones the original method of Fischer* is usually followed. For 1 gm. of sugar, 2 gms. of phenylhydrazine chloride and 3 gms. crystallized sodium acetate ($CH_3COONa + 3\ H_2O$) and 20 c.c. of water are heated together for $\frac{3}{4}$ to $1\frac{1}{2}$ hours in a large test tube of about 50 c.c. capacity placed in a boiling-water bath. The contents of the tube are stirred occasionally to promote crystallization. Instead of the chloride one may employ a solution of phenylhydrazine acetate, prepared by adding concentrated acetic acid drop by drop to phenylhydrazine until the turbid emulsion clears. The osazone reaction with the substituted hydrazines is conducted in the same way as with phenylhydrazine.

The osazones of the sugars are yellowish-colored crystalline compounds of variable solubility. The osazones of the monosaccharides crystallize out from the hot solutions; those of the disaccharides, maltose and lactose, however, separate only after cooling. A separation of the osazones of the mono- and disaccharides can be accomplished in this manner, a second crystallization usually rendering the separation complete. While the osazones of the monosaccharides are nearly all of much lower solubility than the corresponding hydrazones, the osazone separation is never complete.

* Ber., **17**, 579.

*Yield and Time for Formation of Osazones.* — Sugars differ greatly in the amount of osazone which is formed under a definite method of treatment, and this property has been utilized as a means of identification. Maquenne,* for example, has determined the yield of osazones obtained by heating 1 gm. of different sugars in 100 c.c. of water with 5 c.c. of a solution, containing 40 gms. phenylhydrazine and 40 gms. glacial acetic acid in 100 c.c., for 1 hour in a boiling-water bath. The sugars studied by Maquenne are arranged in Table LXV in the order of yield of osazone.

TABLE LXV

*Showing Yield of Osazones and Time of Precipitation for Different Sugars*

| Sugar. | Phenylosazone from 1 gram sugar. | Time for precipitation. |
|---|---|---|
| | Gram. | |
| Sorbose | 0.82 | Turbid in 12 min. |
| Fructose | 0.70 | Precipitate in 5 min. |
| Xylose | 0.40 | Precipitate in 13 min. |
| Glucose | 0.32 | Precipitate in 8 min. |
| Arabinose | 0.27 | Turbid in 30 min. |
| Galactose | 0.23 | Precipitate in 30 min. |
| Rhamnose | 0.15 | Precipitate in 25 min. |
| Lactose | 0.11 | Precipitate only on cooling. |
| Maltose | 0.11 | Precipitate only on cooling. |

It is noted that the ketoses, sorbose and fructose are characterized by a much greater yield of osazone. The theoretical yield of osazone from 1 gm. of sugar is 2.19 gms. for pentoses, 1.99 gms. for hexoses and 1.53 gms. for disaccharides. This shows how large a part of even the more insoluble osazones were unprecipitated in Maquenne's experiments. The latter, however, were not intended to give the conditions of maximum yield and were designed simply for purposes of comparison.

Fischer by heating one part glucose with two parts phenylhydrazine chloride, three parts sodium acetate and 20 parts of water for 1½ hours upon the water bath obtained 85 to 90 per cent of the weight of sugar as osazone. This is nearly three times the amount obtained by Maquenne, but is still less than 50 per cent of the theoretical yield.

Mulliken † has based a scheme for the identification of pure sugars upon the time of separation of the osazones. Fischer's method of making the test is followed, 0.1 gm. sugar, 0.2 gm. pure phenylhydrazine chloride, 0.3 gm. sodium acetate and 2 c.c. water being mixed in a

* Maquenne's "Les Sucres," p. 266; Compt. rend., **112**, 799.

† Mulliken's "Identification of pure Organic Compounds."

small test tube, corked loosely to prevent evaporation and heated in boiling water. The tube is shaken occasionally without removing from the bath and the time noted for the separation of a precipitate. Under the above conditions Mulliken noted the following:

| Sugar. | Time for osazone separation. | Sugar. | Time for osazone separation. |
|---|---|---|---|
| | Minutes. | | Minutes. |
| Fructose | 2 | Arabinose | 1 |
| Sorbose | 3½ | Galactose | 15–19 |
| Glucose | 4–5 | Sucrose | 30 (due to slight inversion). |
| Xylose | 7 | Maltose | No precipitate in hot solution. |
| Rhamnose | 9 | Lactose | No precipitate in hot solution. |

The relation of the sugars as regards time of osazone formation agrees closely with that noted by Maquenne.

Sherman and Williams * give the following time of osazone formation for different quantities of sugar under the conditions followed by Mulliken, but with double the quantity of reagents and water.

*Time for Precipitation of Osazones*

| Weight of sugar taken. | Glucose. | Fructose. | Invert sugar. | Sucrose. |
|---|---|---|---|---|
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.2 | 4–5 | 1¼–1½ | 1½–1⅔ | 31 |
| 0.1 | 5 | 1¾–2 | 2 | 35 |
| 0.05 | 6½ | 2½ | 3 | 78 |
| 0.01 | 17 | 5½ | 6–6½ | No ppt. |
| 0.005 | 34 | 10 | 14 | |
| 0.0025 | 65 | 17 | | |

Sherman and Williams found that with mixtures of different sugars the time of osazone formation was greatly modified. The following results were noted.

*Influence of Maltose on Glucose*

| Weight of glucose. | Weight of maltose. | | | | In absence of maltose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.01 | No. ppt. | 40 | 30 | 22 | 17 |
| 0.02 | 26–28 | | | | 12–13 |

* J. Am. Chem. Soc., **28,** 629.

*Influence of Lactose on Glucose*

| Weight of glucose. | Weight of lactose. | | | | In absence of lactose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.01 | No ppt. | 50 | 32 | 25 | 17 |
| 0.02 | 45–48 | | | | 12–13 |

*Influence of Sucrose on Glucose*

| Weight of glucose. | Weight of sucrose. | | | | In absence of sucrose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.005 | 15–17 | 15–17 | 22 | 30 | 33–39 |
| 0.01 | 14–16 | 16 | 17 | 17 | 17 |
| 0.2 | 9 | | | | 12–13 |

*Influence of Raffinose on Glucose*

| Weight of glucose. | Weight of raffinose. | | | | In absence of raffinose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.005 | 27–30 | 33–37 | 36–38 | 37–39 | 33–39 |

*Influence of Maltose on Fructose*

| Weight of fructose. | Weight of maltose. | | | | In absence of maltose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.01 | 7–8 | $5\frac{1}{2}$–6 | $5\frac{1}{2}$–$5\frac{3}{4}$ | $5\frac{1}{2}$ | $5\frac{1}{2}$ |

*Influence of Lactose on Fructose*

| Weight of fructose. | Weight of lactose. | | | | In absence of lactose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. | Minutes. | Minutes. | Minutes. | Minutes. | Minutes. |
| 0.01 | $9\frac{1}{2}$–10 | $7\frac{3}{4}$ | $6\frac{3}{4}$ | 6 | $5\frac{1}{2}$ |

*Influence of Sucrose on Fructose*

| Weight of fructose. | Weight of sucrose. | | | | In absence of sucrose. |
|---|---|---|---|---|---|
| | 0.2 gram. | 0.1 gram. | 0.05 gram. | 0.01 gram. | |
| Gram. 0.005 | Minutes. $8\frac{1}{2}$ | Minutes. $8\frac{3}{4}$ | Minutes. $9\frac{1}{3}$ | Minutes. $9\frac{1}{4}$ | Minutes. $9\frac{1}{2}$ |

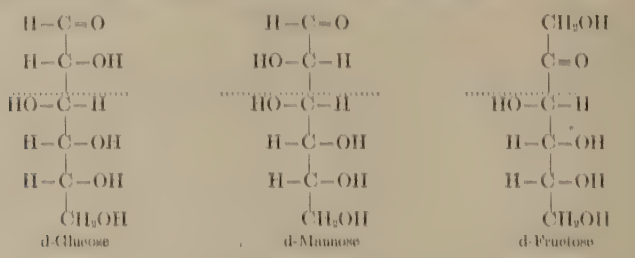
The results show that sucrose accelerates, while maltose and lactose retard the separation of osazone from solutions containing glucose and fructose.

A scheme of identification, based upon yield, or time of formation of osazone under a prescribed method of treatment, is of value only in working with a known quantity of pure sugar. In case of products containing foreign organic and mineral matter, or a mixture of several sugars, the presence of impurities or of other osazones influences crystallization to a very marked degree. This fact prevents the employment of the osazone reaction for exact quantitative purposes.

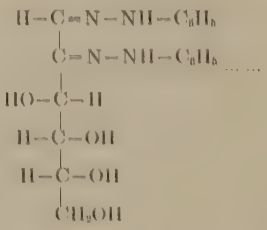
The osazones of sugars after precipitation require to be purified. The crystalline precipitate is filtered off, well washed with cold water, and then pressed as dry as possible between filter paper. The product is then recrystallized from boiling 50 per cent alcohol to which a few drops of pyridine may be added, in case of very insoluble osazones, to promote solubility. Recrystallization may also be effected from acetone and other organic solvents and in case of easily soluble osazones, as of maltose and lactose, from hot water. After dissolving the osazones, the hot solution is filtered and set aside in the cold until crystallization is complete. The purified osazone is then filtered off and dried at a gentle heat. A melting point is then taken which, if the osazone is pure, will remain unchanged after further crystallization. A table of melting points is then consulted and this in many cases is sufficient to identify the osazone. (See Table 24, Appendix.)

*Limitations of the Osazone Reaction.* — The osazone reaction with phenylhydrazine, while invaluable, is not always an absolute test of the identity of a sugar, owing to the fact that a number of isomeric sugars give the same osazone. The pentose sugars d-lyxose and l-xylose, for example, yield the same phenylosazone of melting point 160°–161° C. Similarly the hexose sugars, d-glucose, d-mannose and d-fructose, yield the same phenylosazone of melting point 206° C. In fact any of the isomeric sugars which are mutually transformable (as in contact with

alkalies) give the same osazone. This is made more clear from the following stereoformulæ of glucose, mannose and fructose.




The part of the molecule below the dotted line has the same spatial arrangement in all three sugars. The part of the molecule above the dotted line is the only part of the molecule affected in the osazone reaction, this in all three sugars giving rise to an osazone which has the same structural formula:



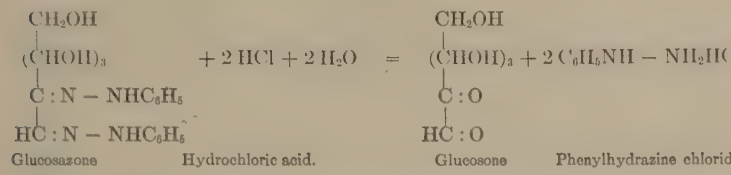

This circumstance, although nullifying the use of phenylosazones in certain cases as a means of identification, has yet thrown a flood of light upon the molecular constitution of sugars.

*Test for Ketoses with Methylphenylhydrazine.* — In distinction from phenylhydrazine the substituted hydrazines do not always give the same osazone reaction with sugars which are mutually transformed. The osazone reaction with substituted hydrazines has, therefore, a distinct qualitative value. Methylphenylhydrazine, for example, forms very readily a characteristic osazone with d-fructose, but does not form an osazone with d-glucose or d-mannose or any of the other aldose sugars. The osazone reaction with methylphenylhydrazine is, therefore, serviceable in distinguishing aldoses from ketoses.

*Decomposition of Osazones into Osones.* — While hydrazones, upon decomposition with strong hydrochloric acid or with benzaldehyde or formaldehyde, yield the component sugar, the osazones cannot be resolved in this manner. The osazone reaction is consequently of value

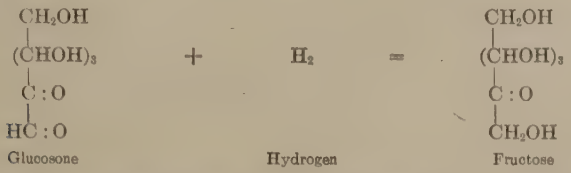
only as a means of identifying and not of separating sugars. The decomposition of osazones with acids and aldehydes has, however, a considerable theoretical interest which may be considered briefly in this connection.

Treatment of osazones with concentrated hydrochloric acid or with certain aldehydes causes, as in the case of hydrazones, a separation of the phenylhydrazine; the product remaining behind, however, is not the original sugar, but a compound with two adjacent carbonyl groups called an osone. The reaction of glucosazone with hydrochloric acid, for example, is:




In case of osazones soluble in hot water the conversion into osones can be easily effected with benzaldehyde in presence of sufficient alcohol, the phenylhydrazine being separated as benzaldehyde-phenylhydrazone and the osone remaining behind in solution.

Osones upon treatment with zinc dust and acetic acid are reduced by the nascent hydrogen to a sugar, the end carbonyl group being converted always to an alcohol group, as shown in the following equation for glucosone.




It will be seen from the above reaction that the sugar obtained by reduction of an osone is always a ketose. By this means glucose and mannose can be transformed into fructose, and this type of reaction is true for the conversion of any aldose into the corresponding ketose, the steps of the transformation being always

Aldose ⟶ Osazone ⟶ Osone ⟶ Ketose.

The osones, while of great service in establishing the relationship of different sugars to one another, have no value either in qualitative or quantitative sugar analysis.

### THE IDENTIFICATION OF HYDRAZONES AND OSAZONES

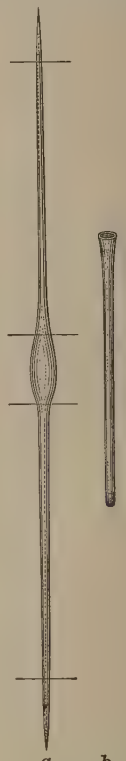
The identification of hydrazones and osazones, by examination of their physical properties, although belonging strictly to the tests for individual sugars, is introduced for convenience at this point.

**Determination of Melting Point of Hydrazones and Osazones.** — The determination of melting point is the principal physical method for identification of hydrazones and osazones.

Fig. 162. — Apparatus for determining melting points.

*Capillary-tube Method.* — The capillary-tube method is the one most generally employed for determining melting points. The essential requirements in way of apparatus are shown in Fig. 162. A long-neck flask with a small body of about 20-c.c. capacity is filled about two-thirds with pure concentrated sulphuric acid; to prevent discoloration of the acid through accidental contamination with organic matter a small crystal of potassium nitrate, the size of a pinhead, is dropped in. The flask is clamped to a lamp-stand in the manner shown. The opening of the flask is fitted with a perforated cork containing a groove upon the side to allow escape of expanding air. The perforation in the cork should be of such a size as to hold a thermometer, graduated to 300° C., tightly in position; the bulb of the latter should be above the bottom of the flask and yet be submerged entirely in the acid.

The capillary tubes for holding the hydrazone or osazone are best prepared by thoroughly softening a piece of glass tubing by turning it in the flame and then drawing it out to about 1 to 1.5-mm. diameter. By continuing this process backwards along the tube a number of sections are obtained similar to Fig. 163*a*; the sections are then filed off at the points indicated and the smaller ends melted together in the flame. Small tubes of the size and shape shown in Fig. 163*b* are thus obtained.



*a* *b*

Fig. 163.— Showing preparation of capillary tubes for determining melting points.

A small amount of finely powdered hydrazone or osazone is then introduced into the open end of the tube and the latter gently tapped until

the substance has settled to the bottom. To prevent the powdered material from forming too loose a layer it is usually well to push it tightly down by means of a platinum-wire or thin-glass rod. The depth of substance in the tube should not exceed 2 mm. The capillary tube containing the substance is then attached to the thermometer either by binding it with a piece of fine platinum wire or by dipping it first in concentrated sulphuric acid and allowing it to stick to the thermometer bulb by adhesion. The tube is placed so that the layer of substance is even with the center of the mercury bulb.

After placing the thermometer and tube in position, as shown in Fig. 162, a small flame is placed beneath the flask and the temperature raised until the liquefaction of the powdered crystals indicates the temperature of melting. Hydrazones and osazones at the point of melting decompose with darkening of color, the evolution of gas causing the liquefied substance to foam upwards in the stem of the tube. The first determination of melting point is only preliminary and a second and third trial should always be made with fresh tubes and material. The acid in the subsequent tests is heated rapidly to about 5° C. below the melting point first observed and then the temperature raised gradually so that the thread of mercury in the thermometer comes to rest just at the point of liquefaction. The entire operation for glucosazone, for example, melting at 204 ° to 205 °C., should not consume over 4 minutes. Undue protraction of the time of heating affects the result of the determination very markedly and the wide discrepancies noted in the literature between melting-point determinations of the same osazone by different authorities are due largely to this cause.

*Maquenne's Block.* — A second method for determining melting points of hydrazones and osazones is employed considerably by French chemists. This method involves the use of the Maquenne Block, an apparatus invented by Maquenne in 1887, the essential features of which are shown in Fig. 164.

The important part of Maquenne's apparatus consists of a prismatic block (*A*) of brass, weighing about 2 kilos, which is placed in a frame with one of its edges resting above the openings of a long gas burner (*B*). In one end of the block about 5 mm. below the upper surface a hole is bored, extending nearly the length of the block, into which a thermometer (*T*) can be inserted. In the upper level surface of the block are a number of small, round cavities. In conducting a determination a small amount of substance is placed in one of the cavities, which, to prevent disturbances from air drafts, is covered with a small glass; the thermometer is then inserted so that its bulb is about underneath the cavity and

the burner started with a low, uniform flame. The temperature is slowly elevated until the substance begins to melt when the thermometer is drawn out or pushed in until just the end of the mercury thread projects and the temperature noted. The block is now cooled slightly and a second determination made more slowly than before, using a cavity above the bulb of the thermometer in its second position. Owing to the fact that the block has nearly the same temperature, the entire column of mercury is brought to the same temperature as that of the melting substance and no correction due to contraction of the thread outside the unheated portion of the thermometer is necessary as by the method of melting-point determination previously described.

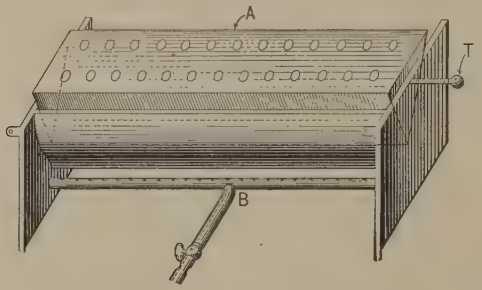



Fig. 164. — Maquenne's block for determining melting points.

A comparison of melting points of glucose-phenylosazone by the two methods shows the following: capillary tube 205° C. (Fischer), Maquenne Block 230° to 232° C. (Bertrand). From this it would appear that the Maquenne Block gives considerably higher melting points than the capillary-tube method. A critical comparison of the two methods by Müther * (see Table LXVI, opposite page) shows, however, that this is not always the case.

It will be seen that Müther obtained for glucosazone results by the block agreeing very closely with those by the tube, the range found by the block being 200° to 206° C. and by the tube 203.5° to 205° C. The greater variation by the block is attributed by Müther to the unequal distribution of heat through the brass, the outer surface being more quickly warmed than the center; differences from 3° to 6° C. were also noted for different positions of the thermometer inside the block. The slowness with which the block is heated and cooled and the difficulty with which the cavities are cleaned are also serious objections. With substances which sublime, the Maquenne Block cannot

* Dissertation, Göttingen, 1903.

be used on account of the rapid condensation of material from the cavity upon the cover glass. These objections together with the high cost of the apparatus (about $15.00, duty free) render it much less desirable for determining melting points than the simpler capillary-tube method.

TABLE LXVI

*Showing Melting Points of Hydrazones and Osazones by Different Methods.* (Müther.)

| Compound. | Method of melting point. | |
|---|---|---|
| | Capillary tube. | Maquenne block. |
| | Deg. C. | Deg. C. |
| Arabinose-methylphenylhydrazone | 164 | 158–160 |
| | | 159–160 |
| | | 159–160 |
| | | 162 |
| Arabinose-diphenylhydrazone | 203–204 | 198 |
| | | 199–200 |
| Fucose-methylphenylhydrazone | 177 | 174–175 |
| | | 172–173 |
| | | 170–171 |
| Fucose-benzylphenylhydrazone | 172–173 | 165–167 |
| | | 173–174 |
| Mannose-phenylhydrazone | 188–189 | 187 |
| | 188–189 | 191–192 |
| Fructose-osazone (glucosazone) | 203.5 | 202–203 |
| | 204–205 | 200–201 |
| | 203.5 | 204–205 |
| | 203–204 | 205–206 |
| | 203.5 | 205 |

*Isomerism and Variability in Melting Points of Hydrazones.* — A peculiarity of a number of hydrazones is the existence of two isomers of different crystalline form, melting point and specific rotation. Thus in case of d-glucose-phenylhydrazone the following properties were noted by Fischer and Tafel,* and by Simon and Bénard.†

| | I. | II. |
|---|---|---|
| Crystalline form | Fine needles. | Long needles. |
| Melting point | 144°–146° | 115°–116° |
| Specific rotation after solution | −66.57 | −15.3 |
| Specific rotation after standing | −52.00 | −52.9 |

It is seen that the isomeric hydrazones each possess mutarotation, and in solution undergo transformation into the same compound.

* Ber., **20,** 2566.

† Compt. rend., **132,** 564.

The isomerism has been attributed to the existence of hydrazones of $\alpha$- and $\beta$-glucose, but the conditions for their separate formation have not been definitely established.

Similar differences have been noted in the case of other hydrazones, but whether the variation in properties is due to isomerism or to a difference in purity is not always certain.

**Optical Activity of Hydrazones and Osazones as a Means of Identification.** — In addition to melting point the optical activity of hydrazones and osazones is sometimes employed as a means of identification.

Owing to the low solubility of some of the compounds and the high color of some of the solutions the polarization of hydrazones and osazones can not always be measured with exactness. In the case of hydrazones the existence of different isomers, as in the case of glucose-phenylhydrazone just cited, may cause wide differences in polarization. Mutarotation, which was noted in the case of glucose-phenylhydrazone, has also been observed with some of the osazones. Thus Allen and Tollens * found for l-arabinose-phenylosazone $[\alpha]_D = +18.9$ after dissolving in alcohol, but after standing a short time the solution became optically inactive.

The rotatory power of hydrazones and osazones also varies greatly for different solvents. Thus Lobry de Bruyn and van Ekenstein † found the following rotations for different $\beta$-naphthylhydrazones in methyl alcohol and glacial acetic acid.

| | Methyl alcohol. | Glacial acetic acid. |
|---|---|---|
| Rhamnose-$\beta$-naphthylhydrazone | + 8.4 | −11.8 |
| Glucose-$\beta$-naphthylhydrazone | +40.2 | 0 |
| Mannose-$\beta$-naphthylhydrazone | +16.8 | 0 |
| Galactose-$\beta$-naphthylhydrazone | +24.8 | + 2 |

For purposes of comparison and identification the rotations of hydrazones and osazones must be measured, therefore, under exactly similar conditions as to quantity of material and nature of solvent. Neuberg ‡ recommends dissolving 0.2 gm. of osazone in a mixture of 4 gms. pyridine and 6 gms. absolute alcohol, and reading the solution in a 200-mm. tube in a polarimeter. The following rotations were obtained by Neuberg for different osazones when working under the above conditions:

* Z. Ver. Deut. Zuckerind., **40**, 1033.
† Rec. Trav. Pays Bas, **15**, 226.
‡ Ber., **32**, 3384.

TABLE LXVII

*Giving Polarization of Different Osazones*

| | |
|---|---|
| l-Arabinose-phenylosazone | +1°10′ |
| l-Arabinose-p-bromophenylosazone | +0°28′ |
| Xylose-phenylosazone | −0°15′ |
| Xylose-p-bromophenylosazone | ±0° |
| Rhamnose-phenylosazone | +1°24′ |
| d-Glucose-phenylosazone | −1°30′ |
| d-Glucose-p-bromophenylosazone | −0°31′ |
| d-Galactose-phenylosazone | +0°48′ |
| Sorbose-phenylosazone | −0°15′ |
| Maltose-phenylosazone | +1°30′ |
| Lactose-phenylosazone | ±0° |

The rotations are small and in some cases uncertain so that this method of identification upon the whole is less satisfactory than a melting-point determination.

In case of the hydrazones and osazones of optically opposite isomeric sugars (which, as regards melting point and solubility, behave alike except in the special case where optically active hydrazines are used), a determination of the optical activity of the compound is the only ready means of identification. Thus Fischer * gives for the phenylhydrazones of d- and l-galactose the following constants.

| | Melting point. | $[\alpha]_D$ |
|---|---|---|
| d-Galactose-phenylhydrazone | 158° | −21.6 |
| l-Galactose-phenylhydrazone | 158° | +21.6 |

Fischer also gives for the phenylhydrazones of d- and l-mannose

| | Melting point. | Rotation. |
|---|---|---|
| d-Mannose-phenylhydrazone | 195° | −1.2 |
| l-Mannose-phenylhydrazone | 195° | +1.2 |

The rotations in the latter case were the angular readings obtained in a 100-mm. tube upon a solution of 0.1 gm. hydrazone in 1 c.c. cold concentrated hydrochloric acid and diluted with 5 c.c. of water.

**Employment of Optically Active Hydrazines for Separating Sugars from Racemic Mixtures.** — Neuberg † has recently employed optically active hydrazines for analyzing racemic mixtures of sugars.

If two optically opposite isomeric sugars ("antipodes") +S and −S form hydrazones with an optically inactive hydrazine H, the resulting compounds, which may be represented by the symbols +SH and −SH are also antipodes, and, although of exactly opposite rotations,

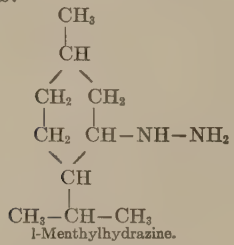
* Fischer's "Untersuchungen über Kohlenhydrate."

† Ber., **36,** 1192; **38,** 866, 868.

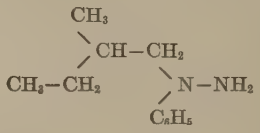
have in other respects, such as specific gravity, melting point, solubility, etc., the same physical properties. A separation of two such hydrazones is consequently not possible by the ordinary methods of analysis.

If, however, the two sugars +S and −S combine with an optically active hydrazine as +H, the resulting hydrazones + S + H and − S + H are not optical antipodes and show well-defined differences in solubility, melting point and other properties. A separation of the two hydrazones is thus made possible by the ordinary methods of fractional crystallization.

The hydrazines, which have been used by Neuberg and his coworkers for this method of separating sugars, are l-menthylhydrazine and d-amylphenylhydrazine, the structural formulæ of which are as follows:



l-Menthylhydrazine.

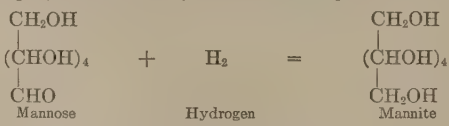


d-Amylphenylhydrazine

The method has been employed successfully by Neuberg in resolving the racemic sugar d,l-arabinose, which occurs in the urine of many persons suffering from pentosuria; d,l-arabinose gives with l-menthylhydrazine an easily soluble l-arabinose-l-menthylhydrazone and a very insoluble d-arabinose-l-menthylhydrazone. The latter is filtered off and upon treatment with formaldehyde (p. 348) is easily decomposed with liberation of the free sugar d-arabinose.

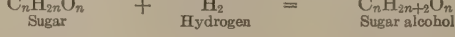
## IV. MISCELLANEOUS REACTIONS OF SUGARS

**Reactions of Sugars with Reducing Agents.**—The simple reducing sugars, in their character of aldehydes or ketones, are easily transformed by reducing agents into the corresponding alcohols. The sugar mannose, for example, is reduced by sodium amalgam to the alcohol mannite.



Mannose — Hydrogen — Mannite

A more general type of equation would be:



Sugar — Hydrogen — Sugar alcohol

The reactions of the different sugars with reducing agents are of comparatively minor importance as regards use in sugar analysis.

A description of the different sugar alcohols, with reactions and methods of identification, is given in Chapter XXIII.

**Reactions of Sugars with Weak Oxidizing Agents.** — Reducing sugars belonging to the aldoses are changed by means of the less powerful oxidizing agents, such as bromine water, into the corresponding monobasic acids. Thus:

$$\underset{\text{Aldo-hexose}}{\begin{matrix}CH_2OH\\|\\(CHOH)_4\\|\\CHO\end{matrix}} + \underset{\text{Bromine water}}{2\,Br + H_2O} = \underset{\text{Hexonic acid}}{\begin{matrix}CH_2OH\\|\\(CHOH)_4\\|\\COOH\end{matrix}} + \underset{\text{Hydrobromic acid}}{2\,HBr}$$

In carrying out the reaction 1 part sugar is treated with 5 parts of water and 2 parts of bromine, and the solution kept at room temperature for 1 to 3 days.

Ketose sugars, upon treatment with bromine water, undergo but little oxidation during the first few days. Prolonged action, or elevation of temperature, will, however, oxidize ketoses with a breaking up of the molecule into several acids of fewer carbon atoms.

*Rate of Oxidation with Bromine as a Test for Aldoses and Ketoses.* — The rate of oxidation of several aldose sugars with bromine water, as compared with fructose, is shown in the following experiments by Votoček and Nemecek;* 0.5 gm. of pure sugar was dissolved in a 50-c.c. flask in 9 c.c. of water, 40 c.c. of bromine water (saturated at room temperature) were then added and the volume made up to 50 c.c. After standing at room temperature (21° C.) for 24 hours, the unoxidized sugar was determined in each flask with the following results:

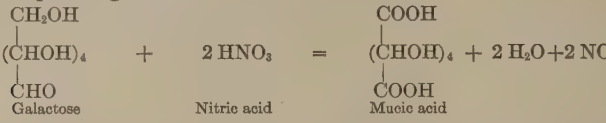
| Sugar. | Per cent sugar unoxidized. | Sugar. | Per cent sugar unoxidized. |
|---|---|---|---|
| d-Galactose | 5.10 | l-Xylose | 25.68 |
| l-Arabinose | 7.56 | Rhamnose | 39.19 |
| d-Glucose | 22.20 | d-Fructose | 100.00 |

Votoček and Nemecek propose their method as a means for distinguishing aldoses from ketoses and also as a method for examining sugar mixtures. In case of the latter the aldoses are oxidized away with bromine water, leaving the ketoses in better condition for isolation.

**Reactions of Sugars with Strong Oxidizing Agents.** — Reducing sugars belonging to the normal unsubstituted aldoses are changed upon

* Z. Zuckerind. Böhmen, **34**, 399.

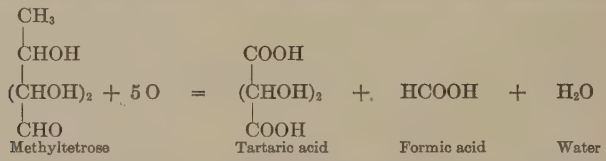
warming with stronger oxidizing agents, as 30 per cent nitric acid, into the corresponding dibasic acids. Thus




In carrying out the reaction one part of sugar is heated with $2\frac{1}{2}$ parts nitric acid of 1.2 sp. gr. and gently warmed at 40° to 50° C. until no more nitrous fumes are evolved. The solution is then heated upon the water bath until all nitric acid is expelled and then evaporated, when the acid or its lactone will in many cases crystallize; when crystallization does not occur separation from impurities is effected by forming an insoluble salt or other derivative from which the acid can afterward be liberated in the pure condition.

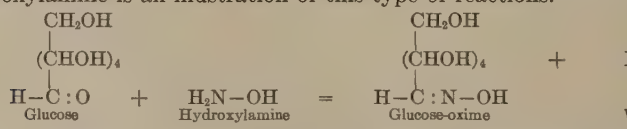
Ketose sugars, upon oxidation with nitric acid, are degraded into lower oxidation products, of which oxalic acid is usually formed in largest amount.

The substituted aldose sugars, as the methyltetroses, methylpentoses, methylhexoses, etc., lose the methyl group upon oxidation with nitric acid and are degraded into dibasic acids of one less carbon atom.



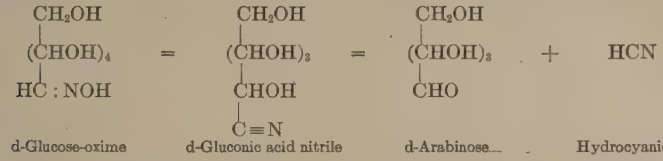

In the same way the methylpentoses, rhamnose, rhodeose and fucose are oxidized into trioxyglutaric acids, the methylhexoses into tetraoxyadipic acids, etc.

**Oxime Reaction of Sugars.** — Many of the reducing sugars react with hydroxylamine, after the manner of all aldehydes and ketones, with formation of oximes. The following combination of glucose with hydroxylamine is an illustration of this type of reactions.



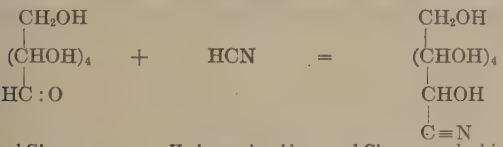

The oximes of the sugars are often difficult to isolate and the reaction, for this reason, has but little value in sugar analysis. In sugar synthesis, however, the oxime reaction has considerable importance, for by its means a monosaccharide may be changed into another sugar con-

taining one less carbon atom. This is done by first making the oxime of the sugar and then heating the latter with acetic anhydride; the resulting acetyl-nitrile derivative is then heated with an ammoniacal solution of silver oxide which splits off the acetic acid and hydrocyanic acid groups with formation of a lower sugar (Wohl's* synthesis). The reaction in its simplest phase is represented as follows:

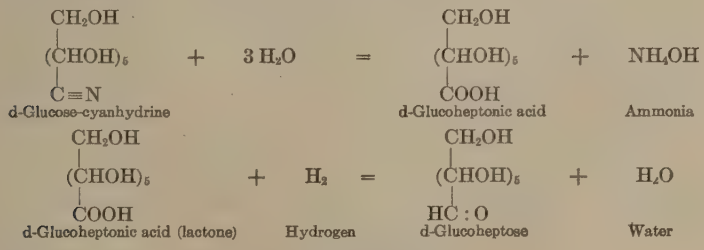



The hexose sugar d-glucose is thus converted into the pentose sugar d-arabinose. In the same manner d-arabinose can be converted into the tetrose sugar d-erythrose.

**Cyanhydrine Reaction of Sugars.** — The reducing sugars, similar to all aldehydes and ketones, react with hydrocyanic acid forming a characteristic group of compounds known as cyanhydrines.



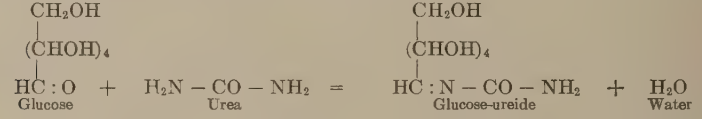

The cyanhydrine reaction, as that of the oximes, while having but little value in sugar analysis, has very great importance in sugar synthesis for by its means a monosaccharide may be built up into another sugar having one more carbon atom. This is done by first making the cyanhydrine, saponifying this to form the corresponding acid, and then reducing the latter with sodium amalgam which produces the corresponding sugar. The formation of glucoheptose from glucose is given as an illustration of this type of reaction.




* Ber., **26**, 730.

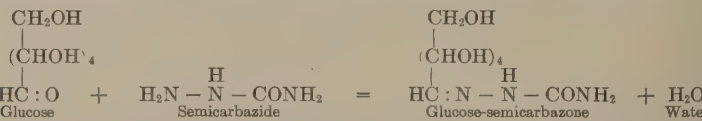
In the same manner, starting from the hexoses, mannose and galactose, mannoheptose and galaheptose can be derived. The heptoses by the same cyanhydrine synthesis have been built up into the corresponding octoses $C_8H_{16}O_8$ and the latter in turn into the corresponding nonoses $C_9H_{18}O_9$. For details as to this method of forming sugars the work of Fischer * should be consulted.

**Ureide Reaction of Sugars.** — Nearly all reducing sugars, with exception of the ketoses, react at moderately warm temperatures with urea in presence of dilute sulphuric or hydrochloric acid to form a group of compounds called ureides. The reaction is analogous to that with phenylhydrazine, the hydrogen of the amino group withdrawing the oxygen from the aldehyde group of the sugar. The reaction with glucose and urea is given by way of example.



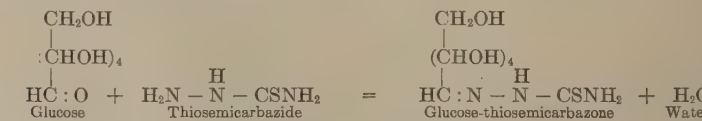

The ureides are partly crystalline and partly amorphous bodies. In aqueous solution they are decomposed upon heating with evolution of ammonia and liberation of the free sugar.

**Semicarbazone Reaction of Sugars.** — Very similar to the reaction of sugars with urea is that with semicarbazide; the latter in alcoholic solution combines with the aldoses to form a group of substances called semicarbazones. The reaction with glucose is given as illustration.




The semicarbazones are well-defined crystalline compounds; when warmed with benzaldehyde in alcohol solution they are decomposed into free sugar with formation of benzaldehyde semicarbazone.

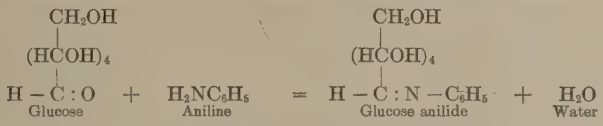
**Thiosemicarbazone Reaction of Sugars.** — Exactly similar to the previous reaction is the behavior of aldose sugars with thiosemicarbazide. The reaction with glucose proceeds as follows:



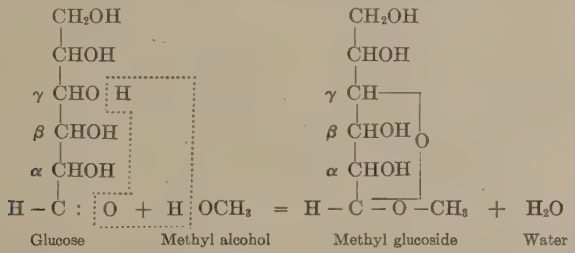

* Ann., **270,** 64; **288,** 139.

The thiosemicarbazones are well-defined crystalline compounds similar in many properties to the semicarbazones.

**Reactions of Sugars with Aromatic Amines.** — The ease with which reducing sugars unite with compounds containing an amino group, as shown in the case of the hydrazones, oximes, ureides, semicarbazones, etc., is further exemplified by the reactions of sugars with different aromatic amines, such as aniline, toluidine, etc. Glucose, for example, reacts with aniline in alcoholic solution as follows:



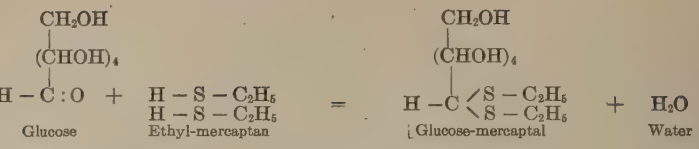

**Reactions of Sugars with Alcohols.** — By leading dry hydrochloric-acid gas into the solution of a reducing sugar in an alcohol the corresponding alcohol derivative of the sugar is formed. The compounds thus prepared are called glucosides from their resemblance to the group of plant substances known under this name. The reaction of glucose with methyl alcohol is given as illustration.




In the same manner glucosides of the other sugars have been made as methyl arabinoside, methyl xyloside, methyl rhamnoside, methyl fructoside, also of the other alcohols as ethyl glucoside, etc. The compounds thus prepared are well-defined crystalline substances, easily soluble in water, do not reduce Fehling's solution and do not react with phenylhydrazine.

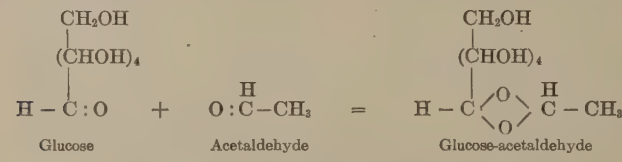
The reactions of the reducing sugars with alcohols are but little used as a means of identification. The synthetic glucosides have, however, a great interest for the sugar chemist in other ways.

**Mercaptal Reaction of Sugars.** — Nearly all reducing sugars, except ketoses, react with the mercaptans in presence of concentrated hydrochloric acid to form mercaptals. The reaction with glucose and ethyl-mercaptan is given as illustration.




The mercaptals of the sugars are well-defined crystalline compounds, soluble in hot water; they do not reduce Fehling's solution and do not react with phenylhydrazine.

**Reactions of Sugars with Aldehydes.** — The simple reducing sugars react with a large number of aldehydes (formaldehyde, acetaldehyde, benzaldehyde, salicylaldehyde, furfural, etc.) to form a variety of condensation products. The latter, for the most part, are of a gummy or sirupy nature and do not crystallize readily. The combination of glucose with acetaldehyde is given as an illustration of this type of reaction.



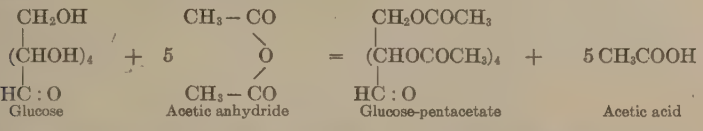

**Reactions of Sugars with Polyvalent Phenols.** — The simple reducing sugars unite with different polyvalent phenols (resorcin, orcin, hydroquinone, phloroglucin, pyrogallol, etc.) to form a series of amorphous ill-defined condensation products. The reaction is carried out in the cold in presence of hydrochloric acid. The following combination of arabinose with resorcin is given as an illustration of this type of reaction.

The condensation products of the sugars with polyvalent phenols when heated with concentrated hydrochloric acid are decomposed, showing the color and spectral reactions characteristic for each class of sugar (see p. 341).

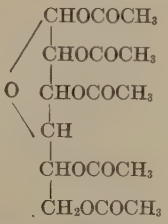
**Reactions of Sugars with Acid Radicals.** — In the many different reactions previously described the aldehyde or ketone group of the sugar molecule is the one mostly involved. In the reactions of sugars with acid radicals, as acetic and benzoic, the alcohol groups of the molecule are affected; the aldehydic characteristics of the sugar are also usually modified in the higher derivatives. The number of acid derivatives obtainable with a sugar is dependent upon the number of

alcohol groups. In the case of hexoses having five such groups there are mono-, di-, tri-, tetra- and penta- acetates and benzoates; with sugars of fewer alcohol groups the number of these combinations is correspondingly less.

*Reaction of Sugars with Acetic Anhydride.* — Acetates of the sugars are formed by heating with acetic anhydride. A mixture of different acetates usually results during the reaction, the separation of these being effected by fractional crystallization or by the use of different solvents. To obtain the highest acetates, the reaction must be carried out in presence of zinc chloride or some other condensing agent. The formation of glucose pentacetate is given as illustration of this type of reaction:




The lower acetates of the sugars are amorphous, easily soluble substances; the higher acetates are crystalline and less soluble in water. By warming with alcoholic potassium or sodium hydroxide, the acetates are all easily saponified with regeneration of the sugar. The lower acetates of the sugars are copper reducing and exhibit other aldehydic properties; the higher acetates, as glucose-pentacetate, lack, however, many aldehyde characteristics, such as formation of hydrazones and oximes. This is probably due to a stable lactonic rearrangement of the molecule as shown by the following formula of Erwig and Königs * for glucose pentacetate.




*Reaction of Sugars with Benzoyl Chloride.* — The acetates of the sugars owing to their solubility are not well adapted for the identification of sugars; the sugar benzoates, however, are marked by a high insolubility in water and their formation is sometimes used as a qualitative test for sugars.

* Ber., **22**, 1464, 2209.

The test, according to the method of Baumann,* is carried out by treating a solution of the sugar with benzoyl chloride in presence of sodium hydroxide; the benzoic radical displaces the H of the hydroxyl groups with formation of sodium chloride and water. A number of benzoates are usually formed in the reaction. In the case of glucose-pentabenzoate the formation proceeds as follows:

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ CHO \end{array} + 5\,C_6H_5COCl + 5\,NaOH = \begin{array}{l} CH_2OCOC_6H_5 \\ | \\ (CHOCOC_6H_5)_4 \\ | \\ CHO \end{array} + 5\,NaCl + 5\,H_2O$$

Glucose — Benzoyl chloride — Sodium hydroxide — Glucose-pentabenzoate — Salt — Water

The Baumann reaction is sufficiently delicate to detect 1 to 2 mgs. glucose in 100 c.c. of water and is sometimes employed for testing urine; 100 c.c. of solution are well shaken with 2 c.c. of benzoyl chloride.

## Special Tests for Reducing Sugars

To the second class of reactions for examining sugars belong the special tests pertaining to group identification; the reactions chosen for description may be divided for convenience into three general classes.

I. Analysis of hydrazones and osazones.
II. Separation of products obtained by decomposition with concentrated hydrochloric acid.
III. Color reactions with phenols in presence of concentrated mineral acids.

### I. Analysis of Hydrazones and Osazones as a Means of Identifying Sugar Groups

If the hydrazone or osazone of a sugar has been separated in a pure condition, an elementary analysis of the compound will serve to identify the group to which the sugar belongs. The osazones, owing to their greater insolubility and ease of preparation, are best adapted for this purpose. The determinations necessary for the identification of an osazone are those of the elements nitrogen and carbon; a determination of hydrogen is also usually included since this element can be determined with little extra trouble at the same time as the carbon determination.

The elementary analysis of osazones and hydrazones is carried out by burning about 0.2 gm. of the substance over cupric oxide in a com-

* Ber., **19**, 3220.

bustion tube. For nitrogen the combustion is carried out by Dumas's method in a current of carbon dioxide after complete displacement of the air. The evolved nitrogen is received in a eudiometer over strong potassium hydroxide solution and its volume measured. From the volume of gas the weight of nitrogen is calculated, making the necessary corrections for atmospheric pressure and temperature.

For carbon and hydrogen the combustion is carried out by Liebig's method in a current of air or oxygen which must be perfectly dry and free from carbon dioxide. The evolved water is collected in weighed tubes, or spirals, containing concentrated sulphuric acid, and the evolved carbon dioxide absorbed in weighed Liebig bulbs containing concentrated potassium hydroxide solution, or in U-tubes filled with soda lime (NaOH + CaO). From the weights of water and carbon dioxide obtained the percentages of carbon and hydrogen are calculated. The percentage of oxygen in osazones and hydrazones is determined by subtracting the sum of the percentages of the other elements from 100.

In the elementary analysis of osazones and hydrazones, as of all other nitrogen compounds, a spiral of copper should be placed in the combustion tube at the exit end in order to effect the reduction of oxides of nitrogen. For complete details as to methods of combustion the chemist is referred to the standard textbooks upon organic analysis.

Having determined the elementary composition of an osazone or hydrazone, reference to a table of percentage composition will usually locate the class of sugar to which the compound belongs. In the following table the formula and percentage composition of phenylosazones are given for various groups of sugars.

| Phenylosazone. | Formula. | Composition. | | | |
|---|---|---|---|---|---|
| | | C per cent. | H per cent. | N per cent. | O per cent. |
| Diose | $C_{14}H_{14}N_4$ | 70.54 | 5.93 | 23.53 | ..... |
| Triose | $C_{15}H_{16}N_4O$ | 67.12 | 6.01 | 20.90 | 5.97 |
| Tetrose | $C_{16}H_{18}N_4O_2$ | 64.39 | 6.08 | 18.80 | 10.73 |
| Pentose | $C_{17}H_{20}N_4O_3$ | 62.16 | 6.14 | 17.08 | 14.62 |
| Methylpentose | $C_{18}H_{22}N_4O_3$ | 63.12 | 6.48 | 16.38 | 14.02 |
| Hexose | $C_{18}H_{22}N_4O_4$ | 60.30 | 6.19 | 15.64 | 17.87 |
| Heptose | $C_{19}H_{24}N_4O_5$ | 58.73 | 6.23 | 14.43 | 20.61 |
| Octose | $C_{20}H_{26}N_4O_6$ | 57.38 | 6.27 | 13.40 | 22.95 |
| Nonose | $C_{21}H_{28}N_4O_7$ | 56.22 | 6.29 | 12.50 | 24.99 |
| Disaccharide | $C_{24}H_{32}N_4O_9$ | 55.35 | 6.20 | 10.77 | 27.68 |

## II. SEPARATION OF PRODUCTS OBTAINED BY DECOMPOSITION WITH CONCENTRATED HYDROCHLORIC ACID AS A MEANS OF IDENTIFYING SUGAR GROUPS

While an elementary analysis of osazones is one of the best means of determining the class to which a sugar belongs there are a number of other special group reactions which are of great value. The most important of these is the separation and identification of some characteristic decomposition product obtained by treating the sugar with concentrated sulphuric or hydrochloric acid. The latter acid is less drastic in its action and is the one most commonly used.

The varied nature of the decomposition products — humus substances, aldehydes, acids, etc. — obtained upon heating sugars with concentrated hydrochloric acid has already been mentioned. It is found, however, that when this treatment is carefully controlled some one characteristic decomposition product will predominate for each particular group of sugar. The following equations, representing ideal types of reaction, are given as illustrations:

I. $C_6H_{12}O_6 = C_5H_8O_3 + HCOOH + H_2O$
(Hexose = Levulinic acid + Formic acid + Water)

II. $C_5H_{10}O_5 = C_5H_4O_2 + 3\,H_2O$
(Pentose = Furfural + Water)

III. $C_5H_9(CH_3)O_5 = C_5H_3(CH_3)O_2 + 3\,H_2O$
(Methylpentose = Methylfurfural + Water)

The above types of reaction hold true not only of the simple sugars above named, but also of the higher saccharides which yield these sugars upon hydrolysis. In fact the initial phase of the reaction in case of the polysaccharides (sucrose, maltose, lactose, raffinose, starch, pentosans, methylpentosans, etc.), is purely hydrolytic, the simple sugars formed being subsequently decomposed after the manner just indicated.

**Levulinic Acid Reaction for Hexose Groups.** — This reaction, which is due to Tollens * and has been extensively studied by his coworkers, has been employed with great success in detecting hexose groups in a large variety of plant and animal substances (cellular tissues of plants, nucleic acids of animal origin, etc.) Owing to the much greater predominance of hexose-producing substances in nature the levulinic acid reaction is usually among the first tests applied in investigating materials of unknown composition.

*Description of Test.* — In carrying out the reaction 5 to 10 gms. of

* Ann., **206,** 207, 226; **243,** 314; Ber., **33,** 1286.

material are treated with 20 to 50 c.c. of hydrochloric acid of 1.09 to 1.10 sp. gr. (18 to 20 per cent) in a flask provided with a rubber stopper and condensing tube, and heated in a boiling-water bath for 5 to 20 hours. The brownish-colored liquid is then cooled and filtered from the precipitate of humus substances; the filtrate is shaken out in a separatory funnel four times with ether, and the ether extract, after pouring through a dry filter, evaporated. The sirupy residue is then gently heated in an open dish to expel the formic acid (see previous equation I). If levulinic acid is present a drop of the sirup dissolved in water in presence of sodium carbonate and iodine will give a precipitate of iodoform, which can also be recognized by its characteristic odor.

The main portion of the sirup is dissolved in water, boiled with an excess of zinc oxide (ZnO), and then, after decolorizing with animal charcoal, filtered and evaporated. The zinc salt of levulinic acid will soon crystallize; the crystals are filtered off, washed with absolute alcohol and ether, and then converted into the silver compound. This is done by dissolving the zinc salt in 5 to 10 c.c. of water, adding silver nitrate slightly in excess of the equivalent amount and heating nearly to boiling, with addition of a little water until the precipitated silver salt has completely dissolved. A little animal charcoal is then added and the solution filtered. The levulinate of silver, $C_5H_7O_3Ag$, which crystallizes will show under the microscope, in case the compound is pure, hexagonal crystals or plates; if the compound is less pure the crystals will be feather-like in appearance. The silver salt is filtered off, washed with cold water, pressed between filter paper and dried in a dark place over concentrated sulphuric acid. The per cent of silver in the salt is determined by strongly igniting a weighed portion in a porcelain crucible. The theoretical amount is 48.39 per cent Ag.

The yield of levulinic acid obtained by treating hexose sugars with hydrochloric acid will vary greatly according to the time of heating and other conditions of the experiment. Conrad and Guthzeit * obtained upon heating 10.5 gms. each of fructose, glucose, and galactose, with 50 c.c. of acid (containing 4.87 gms. HCl gas) for 17 hours the following yield of products.

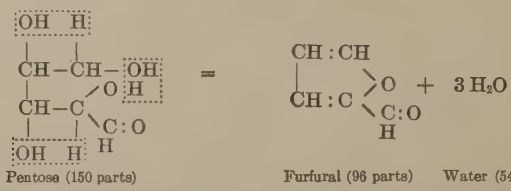
| Sugar. | Humus. | | Levulinic acid. | | Formic acid. | |
|---|---|---|---|---|---|---|
| | Grams. | Per cent. | Grams. | Per cent. | Grams. | Per cent. |
| Fructose | 2.12 | 20.19 | 4.09 | 38.95 | 1.73 | 16.48 |
| Glucose | 1.00 | 9.52 | 3.12 | 29.71 | 1.35 | 12.86 |
| Galactose | 1.77 | 16.86 | 2.85 | 27.14 | 1.11 | 10.57 |

* Ber., **19**, 2575.

From these results it appears that of the three hexose sugars fructose gives the largest yield of levulinic acid and galactose the least. That this is due largely to the greater resistance of glucose and galactose toward the acid was shown by the fact that at the end of the above experiments considerable quantities of these sugars were still undecomposed (in case of glucose 26 per cent). The yield of levulinic acid is too variable for the method to be of any quantitative value.

**Furfural Reaction for Pentose Groups.** — This reaction, which is also due to Tollens,* has been of the greatest value not only as a means of detecting the presence of pentose carbohydrates but also as a means of their quantitative estimation.

The reaction of the pentose sugars with hydrochloric acid proceeds much more nearly according to the equation (II, p. 372) than the reaction of the hexoses, the formation of humus substances being correspondingly less. The following graphic equation shows the decomposition of a pentose sugar into furfural.




Pentose (150 parts) Furfural (96 parts) Water (54 parts)

The theoretical yield of furfural, according to the above equation, is 64 per cent; actual determinations of the furfural, obtained by distilling weighed amounts of the pentose sugars, arabinose and xylose, with hydrochloric acid, give about 47 per cent in case of arabinose and about 57 per cent in case of xylose — yields which are about 75 per cent and 90 per cent respectively of the theoretical.

*Description of Test.* — In carrying out the qualitative test about 5 gms. of substance are heated in a distillation flask with 100 c.c. of hydrochloric acid of 1.06 sp. gr. and successive portions of about 30 c.c. distilled into a receiver, new portions of acid being added to the flask for each quantity distilled. The distillates are then tested for the presence of furfural; the latter in large amounts can usually be detected by its pleasant aromatic odor somewhat resembling that of bitter almond oil. The presence of very small amounts of furfural is best indicated by Schiff's reaction with aniline or xylidine acetate. Aniline acetate reagent is best prepared according to Tollens by mixing in a test tube equal volumes of aniline and water and then adding with constant shak-

* Landw. Vers.-Stat., **39**, 425.

ing glacial acetic acid drop by drop until the milky solution becomes clear. Test paper is prepared by moistening strips of filter paper with the aniline-acetate solution. Application of a drop of distillate containing furfural, even in minute traces, will cause the aniline-acetate paper to turn a bright cherry red.

The presence of furfural in the distillate may also be indicated by first neutralizing the acid solution with sodium carbonate and then adding a solution of phenylhydrazine acetate and stirring. Furfural if present is precipitated as furfural-phenylhydrazone, $C_4H_3OCHN_2HC_6H_5$, which melts at 97° to 98° C.

A better precipitating agent for furfural than phenylhydrazine is phloroglucin. A solution of this compound in hydrochloric acid when added to a distillate containing furfural will cause an immediate darkening of the solution with final precipitation of furfural-phloroglucide, according to equation:

$$\underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Phloroglucin}}{C_6H_6O_3} = \underset{\text{Furfural-phloroglucide}}{C_{11}H_8O_4} + \underset{\text{Water}}{H_2O}$$

*Limitations of Furfural Reaction for Pentoses.* — While all carbohydrates containing a pentose group yield large amounts of furfural upon distillation with hydrochloric acid, it must also be borne in mind that other substances have the same property. All hexose carbohydrates such as starch, cellulose, sucrose, glucose, etc., give small amounts of furfural upon distillation with hydrochloric acid but the yield is too small to interfere seriously with the test for pentoses. Two substances, however, of a non-pentose nature are especially marked by their property of yielding furfural upon distilling with acids and hence require brief mention. These are glucuronic acid and oxycellulose.

Glucuronic acid is an aldehyde-acid derivative of glucose and has the formula $COH(CHOH)_4\,COOH$. By the action of putrefactive bacteria it is converted into the pentose sugar l-xylose.

$$\underset{\text{Glucuronic acid}}{C_6H_{10}O_7} = \underset{\text{Xylose}}{C_5H_{10}O_5} + \underset{\text{Carbon dioxide}}{CO_2}$$

The intimate relationship of glucuronic acid to the pentoses is also shown by the reaction upon distilling with hydrochloric acid.

$$\underset{\text{Glucuronic acid}}{C_6H_{10}O_7} = \underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Water}}{3\,H_2O} + \underset{\text{Carbon dioxide}}{CO_2}$$

Glucuronic acid is sometimes found in the urine, especially after the ingestion of chloral, menthol, camphor, turpentine, acetanilide, alkaloids and many other compounds. Under such conditions a combination takes place in the animal organism between the ingested compound

and the glucuronic acid, the latter apparently being formed as an oxidation product of glycogen. The glucuronic-acid derivative, which is excreted in the urine, may be mistaken for a pentose sugar if the chemist relies solely upon such tests as the furfural reaction and reduction of metallic salt solutions.

One means of determining the presence of glucuronic acid is by means of p-bromophenylhydrazine, which was found by Neuberg * to give a characteristic glucuronic-acid derivative, $C_{12}H_{17}O_7N_2Br$. The exact nature of the compound, whether hydrazone or hydrazide, was not determined. The solution to be tested is heated in a water bath at 60° C. with 5 gms. of p-bromophenylhydrazine chloride and 6 gms. of sodium acetate. If glucuronic acid is present yellowish needle-like crystals will separate in 5 to 10 minutes. The solution is cooled, the crystals filtered off and the filtrate again heated as before; a second crop of crystals may thus be obtained which are filtered off again and the process continued until no more crystals form. The combined precipitates are thoroughly washed with warm water and then with absolute alcohol. Recrystallized from 60 per cent alcohol the crystals melt at 236° C. The crystals dissolved in a mixture of 6 c.c. absolute alcohol and 4 c.c. pyridine have a strong levorotation, $[\alpha]_D = -369$.

Spectroscopic methods for distinguishing between pentoses and glucuronic acid will be described under the color reactions for sugar groups.

Cellulose, when treated with different oxidizing agents, such as nitric acid, chromic acid, hypochlorous acid and permanganate, undergoes a partial oxidation. The oxycellulose derivatives formed under such conditions have the property of yielding furfural upon distillation with hydrochloric acid.

According to the researches of Tollens and Faber † oxycelluloses consist of mixtures of cellulose $(C_6H_{10}O_5)_n$ in different porportions with an oxy-derivative celloxin $(C_6H_8O_6)_n$. The greater the amount of celloxin in the oxycellulose the greater the yield of furfural upon distillation with hydrochloric acid. Cotton, for example, upon treatment with nitric acid at 100° C. for different periods of time, gave the following results:

| Time of treatment. | Composition. | Yield of furfural. |
|---|---|---|
| Hours. | | Per cent. |
| $2\frac{1}{2}$ | $4\,C_6H_{10}O_5,\ C_6H_8O_6$ | 2.3 |
| 4 | $3\,C_6H_{10}O_5,\ C_6H_8O_6$ | 3.2 |

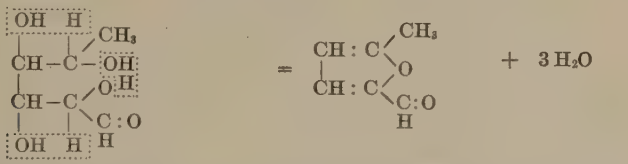
* Ber., **32**, 2395.

† Ber., **32**, 2589.

The yield of furfural calculated to pure celloxin (which has not as yet been isolated) is about 12 per cent.

The oxycelluloses are widely distributed in nature and if reliance is based exclusively upon the furfural reaction erroneous conclusions may be formed as to the occurrence of pentose carbohydrates in plant materials. The oxycelluloses may be easily distinguished, however, from pentosans by the fact that they yield glucose exclusively upon hydrolysis with acids, the hydrolytic products giving none of the reactions (osazone, color tests, etc.) characteristic of the pentoses.

**Methylfurfural Reactions for Methylpentose Groups.** — In the same way that all substances containing pentose groups yield furfural upon distilling with hydrochloric acid, those materials containing methylpentose groups yield methylfurfural. The reaction is perfectly analogous to that described upon page 374.



Methylpentose (164 parts) Methylfurfural (110 parts) Water (54 parts)

The theoretical yield of methylfurfural from methylpentose according to the above reaction is 67.07 per cent. In actual distillation experiments with the methylpentoses, fucose and rhamnose, only from 35 to 40 per cent methylfurfural is obtained or 50 to 60 per cent of the theoretical amount.

In testing natural products for the presence of methylpentose groups, the material is distilled with hydrochloric acid of 1.06 sp. gr. in exactly the same manner as described for pentoses and the distillate tested for methylfurfural. If no furfural is present in the distillate the presence of methylfurfural will be indicated by aniline-acetate paper, which in this instance is colored yellow. If pentosans are also present in the plant material being examined, as is nearly always the case, the presence of furfural in the distillate will color the aniline-acetate paper red and completely mask the yellow color of the methylfurfural reaction. Other tests must, therefore, be employed to detect the presence of methylfurfural.

Maquenne * has devised a reaction by which 1 part methylfurfural can be detected in presence of 9 parts furfural. A small amount of the solution to be tested is added to a mixture containing 3 volumes

* Compt. rend., **109**, 573.

95 per cent alcohol and 1 volume concentrated sulphuric acid and the whole gently warmed. The development of a bright grass-green color throughout the body of the solution indicates the presence of methylfurfural.

Spectral reactions for methylfurfural will be described in a succeeding section.

**Reactions for Tetrose and Triose Groups.** — Excepting the hexoses, pentoses and methylpentoses, but few experiments have been made concerning the reactions of other sugar groups with hydrochloric acid.

Experiments of Tollens and Ellett * show that l-erythrose is decomposed upon heating with hydrochloric acid into lactic acid. The reaction may proceed as follows:

$$\underset{\text{Tetrose}}{C_4H_8O_4} = \underset{\text{Lactic acid}}{C_3H_6O_3} + \underset{\text{Formaldehyde}}{CH_2O}$$

Tollens and Ellett suggest that the above may be a general reaction for tetrose groups, just as levulinic acid is formed from hexoses, furfural from pentoses, and methylfurfural from methylpentoses.

The formation of considerable methylglyoxal $CH_3-CO-COH$ by heating dioxyacetone, $C_3H_6O_3$, with sulphuric acid has been observed by Pinkus.† This may perhaps be a group reaction of trioses.

Further investigations require to be made upon the tetroses and trioses before any results from the above observations can be applied to sugar analysis.

## III. COLOR AND SPECTRAL REACTIONS AS A MEANS OF IDENTIFYING SUGARS

A study of the color reactions and absorption spectra which solutions of different sugars give with various phenols as $\alpha$-naphthol, orcin, resorcin, naphthoresorcin and phloroglucin, in presence of concentrated sulphuric or hydrochloric acids offers frequently a most rapid as well as most reliable method for detecting sugar groups.

**Color Reactions of Ketoses.** — Reference has already been made (p. 340) to the greater ease with which solutions of ketoses show coloration phenomena in contact with concentrated sulphuric acid. The same fact has been noted with the colorations produced with sugars and $\alpha$-naphthol and sulphuric acid, and this has been utilized as one means of detecting the presence of ketose sugars in mixtures.

*$\alpha$-Naphthol Test.* — Pinoff ‡ has modified the $\alpha$-naphthol test for sugars by using a mixture of 750 c.c. 96 per cent alcohol and 200 gms. concentrated sulphuric acid as the condensing agent. By treating in a test

* Ber., **38,** 499. † Ber., **31,** 31. ‡ Ber., **38,** 3314.

tube 0.05 gm. of sugar with 10 c.c. of the alcohol-acid mixture and 0.2 c.c. of alcoholic $\alpha$-naphthol (5 gms. $\alpha$-naphthol dissolved in 100 c.c. 96 per cent alcohol) and heating in boiling water, Pinoff obtained red colorations which in case of sugars containing ketone groups appeared almost immediately; with the aldose sugars 20 minutes or more elapsed before coloration developed. The following table for 11 different sugars by Pinoff gives the time of heating before coloration, the number of absorption bands shown by the solution before the spectroscope and the position of the bands with reference to the wave length of the light absorbed.

TABLE LXVIII

*Giving Absorption Spectra of Sugars with $\alpha$-Naphthol and Sulphuric Acid in Alcohol*

| Sugar. | Time for development of color. | Number of absorption bands. | Wave length in $\mu\mu$ and position of bands. |
|---|---|---|---|
| | Minutes. | | |
| Arabinose | 20 | .. | ..... |
| Rhamnose | 20 | 1 | 562.5 (in yellow) |
| Glucose | 35 | 1 | 532.5 (between yellow and green) |
| Mannose | 31 | 1 | 532.5 (between yellow and green) |
| Galactose | 31 | 1 | 532.5 (between yellow and green) |
| Fructose | 1 | 2 | 573.6 (in yellow), 508.8 (in green) |
| Sorbose | 1 | 2 | 573.6 (in yellow), 508.8 (in green) |
| Sucrose | 1 | 2 | 573.6 (in yellow), 508.8 (in green) |
| Lactose | 31 | 1 | 532.5 (between yellow and green) |
| Maltose | 31 | 1 | 532.5 (between yellow and green) |
| Raffinose | 1 | 2 | 573.6 (in yellow), 508.8 (in green) |

It will be noted that for the ketose sugars fructose and sorbose and for the di- and tri-saccharides sucrose and raffinose, which give the ketose sugar fructose upon hydrolysis, a red coloration is obtained in 1 minute, while for the other sugars 20 to 35 minutes must elapse before coloration. By diluting the 10 c.c. of sulphuric-acid alcohol mixture with 10 c.c. of 96 per cent alcohol before making the test, Pinoff obtained no coloration sufficient to show absorption bands with any of the aldose sugars. For the ketose sugars he obtained the following results:

| Sugar. | Time for development of color. | Number of bands. | Wave length in $\mu\mu$ and position of bands. |
|---|---|---|---|
| | Minutes. | | |
| Fructose | 13 | 1 | 508.8 (in green) |
| Sorbose | 30 | 1 | 508.8 (in green) |
| Sucrose | 15 | 1 | 508.8 (in green) |
| Raffinose | 19 | 1 | 508.8 (in green) |

While diluting the acid-alcohol mixture has practically eliminated the aldoses from the reaction, it has also materially lessened the sensibility of the test for the ketoses.

*Resorcin Test.* — The most convenient color test for distinguishing ketose from aldose sugars is the color reaction with resorcin and hydrochloric acid — generally known as Seliwanoff's * test. The test was originally regarded as peculiar to fructose, but later experiments have shown that it is given by sorbose, tagatose, the keto-pentoses and all other sugars having a ketone group.

The reaction is carried out by mixing in a test tube 10 c.c. of the clarified solution to be tested with 10 c.c. of 25 per cent hydrochloric acid, then adding a little resorcin (about the tip of a knifebladeful), and heating gently over a small flame. If fructose or other ketose is present a fiery eosin-red color will develop, which upon cooling and standing will deposit as an amorphous powder mixed with humus decomposition products.

If the acid solution is made alkaline with soda and then shaken with amyl alcohol, the red coloring matter is dissolved with a greenish fluorescence. If a few drops of absolute alcohol be now added the color becomes a beautiful rose red.

If the red-colored solutions obtained by Seliwanoff's reaction be examined before the spectroscope a distinct absorption band will be noted in the blue near the *F* line. (See Fig. 165.)

It is important in making the test with resorcin that an excess of hydrochloric acid be avoided. The percentage of acid in the final mixture should be about $12\frac{1}{2}$ per cent. If too much strong acid is present, glucose and other aldoses will also react with resorcin and form pink-colored solutions; the latter, while lacking the intensity of color obtained with the ketoses, may nevertheless lead to erroneous conclusions. The resorcin reaction obtained with glucose may be due to a slight transformation of this sugar into fructose. Ost, as a matter of fact, has succeeded in effecting such a transformation by treating glucose in the cold with strong sulphuric acid.

Pinoff † has modified the resorcin test for ketoses by using the alcohol-sulphuric-acid mixture previously described as the condensing agent. In making the test 0.05 gm. of sugar was treated in a test tube with 5 c.c. of the alcohol-sulphuric-acid reagent, 5 c.c. alcohol and 0.2 c.c. of a 5 per cent resorcin solution and the mixture placed in boiling water. The following table for 11 different sugars by Pinoff gives the length of time required for development of color, the number of

* Ber., **20,** 181. † Ber., **38,** 3314.

absorption bands and the position of the bands with reference to wave length of light absorbed.

TABLE LXIX

*Giving Absorption Spectra of Sugars with Resorcin and Sulphuric Acid in Alcohol*

| Sugar. | Time for development of color. | Number of absorption bands. | Wave lengths in $\mu\mu$ and position of bands. |
|---|---|---|---|
| | Minutes. | | |
| Arabinose | 35 | .. | ..... |
| Rhamnose | 35 | .. | ..... |
| Glucose | 32 | 1 | 487.5 (in blue) |
| Mannose | 35 | .. | ..... |
| Galactose | 35 | .. | ..... |
| Fructose | 1 | 1 | 487.5 (in blue) |
| Sorbose | 1 | 1 | 487.5 (in blue) |
| Sucrose | 1 | 1 | 487.5 (in blue) |
| Lactose | 32 | 1 | 487.5 (in blue) |
| Maltose | 32 | 1 | 487.5 (in blue) |
| Raffinose | 1 | 1 | 487.5 (in blue) |

*Naphthoresorcin Test.* — Tollens and Rorive* have employed in place of resorcin naphthoresorcin or 1 : 3 dioxynaphthalin. The ketose sugars fructose and sorbose and the di- and trisaccharides sucrose and raffinose show upon heating with a little naphthoresorcin in presence of hydrochloric acid (1 vol. acid 1.19 sp. gr. and 1 vol. water) beautiful red-colored solutions which show a weak absorption band in the green. The sensibility of this test is about the same as that obtained in Seliwanoff's reaction, but the color has more of a violet tinge than the fiery red obtained with resorcin. The red-colored solutions obtained with naphthoresorcin soon become turbid with formation of a deposit. If the latter is filtered off and dissolved in alcohol a yellowish-brown solution with green fluorescence is obtained which shows a weak absorption band in the green.

**Color Reactions of Pentoses (and Glucuronic Acid).** — The pentoses are distinguished above all other sugar groups for the depth and variety of the color reactions obtained with different polyvalent phenols in presence of concentrated hydrochloric acid. Phloroglucin, orcin and naphthoresorcin are the three compounds most used for this purpose, and the reactions for each of these will be described in the order named.

*Phloroglucin Test.* — Ihl † discovered that solutions of the pentose sugars, or of hydrolytic products derived from substances containing

* Ber., **41**, 1783. † Chemiker Ztg. (1885), 231.

pentosans, gave, upon heating with an equal volume of concentrated hydrochloric acid and a little phloroglucin, a beautiful violet-red color. The colored solution thus obtained when viewed before the spectroscope was found by Tollens and Allen * to show a sharp black absorption band in the yellow of the spectrum between the *D* and *E* lines.

The violet-red solution obtained in the phloroglucin reaction for pentoses soon becomes turbid with deposition of a dark-colored precipitate. If the turbid solution is allowed to stand 3 to 5 minutes, then cooled, filtered and the precipitate washed with cold water on a small rapid filter and then dissolved in 95 per cent alcohol, a permanent red solution is obtained which is perfectly adapted to the study of absorption spectra. If the color is too deep it can be reduced by careful dilution with 96 per cent alcohol. (Tollens's "absatz" method.)

The same color reaction of the pentoses with phloroglucin and hydrochloric acid is given by glucuronic acid and its derivatives, but *not* by oxycellulose. The test, therefore, while enabling the chemist to distinguish between such furfural-yielding substances as pentosans and oxycellulose, does not permit the distinction between glucuronic acid and pentoses (as for example in urine).

*Orcin Test.* — If the reaction for the pentoses just described be carried out with orcin in place of phloroglucin a violet-blue coloration is obtained. The solution, however, becomes rapidly turbid with deposition of a bluish-colored flaky precipitate. If the latter is filtered off and dissolved in alcohol by Tollens's "absatz" method a blue-colored solution is obtained which shows before the spectroscope a very sharp dark band almost exactly over the *D* line of the spectrum. The same reaction is also obtained with glucuronic acid.

Bial † has made the orcin reaction more sensitive by carrying out the test in presence of a little ferric chloride. In this manner it is found possible to distinguish between pentoses and glucuronic acid.

Bial's orcin reagent is prepared by dissolving 1 gm. orcin in 500 c.c. hydrochloric acid of 1.15 sp. gr. (30 per cent) to which 20 drops of an officinal solution of ferric chloride (liquor ferri sesquichloridi) are added.

In making the test 4 to 5 c.c. of the reagent are heated in a test tube to boiling; the solution is removed from the flame and a few drops (never over 1 c.c.) of the solution to be tested added. If pentoses are present a vivid green color will develop almost immediately; the reaction is not given under the above conditions with glucuronic acid.

* Ann., **260,** 289.

† Biochem. Zeitschrift., **3,** 323.

Bial's test has been studied and generally confirmed by Sachs,* and also by Tollens and Lefevre.† The last-named authorities found that a dilute solution of glucuronic acid produced no perceptible coloration under the conditions prescribed by Bial, but that if the solution was heated for any length of time a green color speedily developed. The cause of the retardation is explained by the slower decomposition of glucuronic acid by hydrochloric acid as compared with the pentoses; such a difference in the rate of decomposition is also noted between the pentose sugars themselves, xylose, for example, giving a coloration with Bial's reagent in a shorter time than arabinose.

The green solution obtained by Bial's reaction shows before the spectroscope a dark absorption band in the red between the lines *B* and *C* and a second band in the yellow covering the position of the *D* line of the spectrum.

*Naphthoresorcin Test for Pentoses and Glucuronic Acid.* — Tollens and Rorive ‡ have found that when solutions of different sugars are heated with a little naphthoresorcin in presence of an equal volume of concentrated hydrochloric acid (1.19 sp. gr.) characteristic colored solutions and deposits are formed.

With the pentoses arabinose and xylose a red color develops on heating followed by a bluish turbidity. The deposit dissolves in alcohol to a reddish-brown solution with beautiful green fluorescence, showing a weakly-defined absorption band in the green.

Glucuronic acid gives with naphthoresorcin and hydrochloric acid a bluish turbid solution with blue deposit. The alcoholic solution of the latter is a beautiful blue, only slightly fluorescent, and shows a dark absorption band in the yellow covering the *D* line of the spectrum.

The naphthoresorcin test for glucuronic acid has been improved by Tollens § in the following way. The deposit of coloring matter is treated with ether instead of alcohol; if glucuronic acid is present the ether is colored a violet blue and shows before the spectroscope an absorption band in the yellow, its center lying a little to the right of the *D* sodium line (i.e., toward the green).

The naphthoresorcin deposits obtained with sugars (pentoses, hexoses, etc.) in presence of hydrochloric acid are insoluble in ether and so do not appear in the reaction. The presence of sugar and also of foreign organic matter, as in urine, may change the color of the ether solution from the violet blue characteristic of pure glucuronic acid to a

* Biochem. Zeitschrift., **1**, 384.
† Ber., **40**, 4520.
‡ Ber., **41**, 1783.
§ Ber., **41**, 1788.

violet, red, or reddish brown. The characteristic absorption band in the yellow part of the spectrum will not, however, be interfered with.

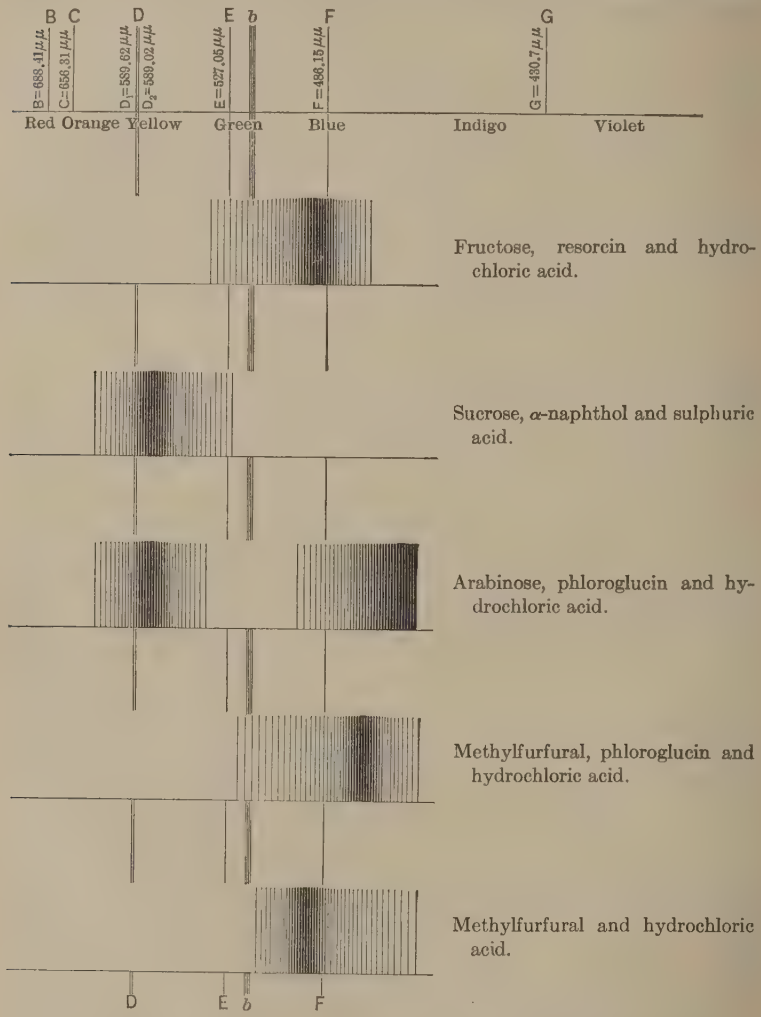



Fig. 165. — Absorption spectra given by different sugars.

The naphthoresorcin test as prescribed by Tollens is made as follows: 5 to 6 c.c. of the solution (urine, etc.) to be tested are treated in

a 16 mm. wide test tube with $\frac{1}{2}$ to 1 c.c. of a 1 per cent solution of naphthoresorcin in alcohol and an equal volume of hydrochloric acid of 1.19 sp. gr. added. The solution is carefully heated to boiling and then kept for 1 minute over a small flame. The dark-colored solution is set aside for 4 minutes and then cooled under a stream of cold water; an equal volume of ether is then added and the whole thoroughly shaken. After the acid solution has settled the ether layer will be colored blue or bluish violet to red, in case glucuronic acid is present, and, if the tube is held before the spectroscope, will show the characteristic absorption band near the *D* line. In case the ether does not separate readily a drop or two of alcohol will hasten the process. If the ether solution is too deeply colored for spectroscopic examination more ether is added until the color is reduced and the unabsorbed part of the spectrum made visible.

The naphthoresorcin deposits of the pentoses and other sugars being insoluble in ether separate as a layer between the colored ether and the lower acid solution.

**Color Reactions of Methylpentoses.** — The color reactions for detection of methylpentoses may be divided into two classes: (1) color reactions made upon the distillate obtained by distilling methylpentoses or methylpentosans with hydrochloric acid; (2) color reactions made directly upon these substances without distillation. The color reactions of the first class are in reality color reactions of methylfurfural to which reference has already been made. It remains, however, to describe some of the spectral reactions of methylfurfural.

*Spectral Reactions of Methylfurfural.* — Tollens and Widtsoe * have detected the presence of methylfurfural in the hydrochloric acid distillate from various plant materials by mixing a few cubic centimeters of the solution with an equal volume of concentrated hydrochloric acid and gently warming. If the solution is colored yellow methylfurfural is present. The yellow solution viewed before the spectroscope will show a dark absorption band between the green and blue of the spectrum near the *F* line. If much methylfurfural is present the band will gradually darken and broaden, the increase in width extending toward the violet and leaving the green unaffected. With considerable methylfurfural the violet end of the spectrum is completely extinguished, the green, however, always remaining clear and transparent. Furfural does not give this reaction although it may affect the delicacy of the test if present in large amount. The reaction, however, will indicate 1 part of methylfurfural in presence of 64 parts furfural ($\frac{1}{32}$ drop methylfurfural

* Ber., **33**, 146.

in presence of 2 drops furfural in 10 c.c. of hydrochloric acid). By use of this test Tollens and Widtsoe were able to detect methylpentosans in different gums, sea weed, leaves of different kinds of trees and a large variety of other plant substances.

Tollens and Oshima * have rendered the spectral reaction for methylfurfural more sensitive by carrying out the test in presence of phloroglucin; 5 c.c. of the hydrochloric acid distillate are treated with 5 c.c. of concentrated hydrochloric acid and a few cubic centimeters of a solution of phloroglucin (in hydrochloric acid of 1.06 sp. gr.) added. After 5 minutes the solution is filtered from the greenish-black precipitate of furfural phloroglucide; if the filtrate is colored yellow or reddish yellow methylfurfural is present. The solution gives before the spectroscope a dark absorption band in the blue. On long standing the solution deposits a red precipitate of methylfurfural phloroglucide which is readily distinguished from the dark-green furfural compound. Absorption spectra of methylfurfural are shown in Fig. 165.

The vivid color reactions of the pentoses with orcin and phloroglucin are not obtained with the methylpentoses. Naphthoresorcin, however, was found by Tollens and Rorive to give a deposit of coloring substance with the methylpentoses, rhamnose and fucose, when heated in presence of hydrochloric acid. The alcoholic solution of the deposits showed a violet blue color with an exceedingly brilliant green fluorescence, which showed before the spectroscope an absorption band in the yellow over the *D* line and a second band in the green.

There are a number of other color spectral reactions which have not been described; these belong, however, more to the reactions of individual sugars and will be given under the description of these.

A few characteristic absorption spectra, useful in testing for sugars, are shown in Fig. 165.

### Reactions of the Non-reducing Sugars

The comparatively small number of sugars, which do not reduce Fehling's solution, all belong to the higher di-, tri- and tetrasaccharides and include sucrose, trehalose, raffinose, melezitose, gentianose, lactosinose, secalose, lupeose and stachyose. The soluble polysaccharides, such as dextrin, inulin, glycogen, etc., although not classified as sugars, are sometimes included for convenience in the group of non-reducing saccharides.

A free aldehyde, or ketone group, to which the reducing sugars owe their peculiar reactivity in the formation of hydrazones, oximes, ureides,

* Ber., **34**, 1425.

mercaptals, etc., is lacking in the non-reducing sugars, and the inability of the latter to reduce Fehling's solution, or to react with phenylhydrazine, dilute alkalies, hydroxylamine, etc., is thus explained.

The non-reducing sugars give many of the color and spectral reactions of the reducing sugars, sucrose and raffinose, for example, giving the $\alpha$-naphthol reaction with sulphuric acid and Seliwanoff's reaction with resorcin and hydrochloric acid. But as previously explained these reactions are not given by the original non-reducing sugar, but by the reducing sugars derived from this by the hydrolytic action of the acid used in making the test.

A carefully controlled hydrolysis by means of acids or enzymes, combined with quantitative measurements of changes in polarization or in copper-reducing power, is the most reliable test for the presence of non-reducing sugars. Methods involving this principle have been described under the inversion methods for determining sucrose and raffinose, and other examples will be given under quantitative chemical methods. Individual tests will be described under the heading of each single sugar in Part II of this Handbook.

# CHAPTER XIV

## REDUCTION METHODS FOR DETERMINING SUGARS

The principal chemical methods for determining sugars are based upon the property which all aldehydes and ketones have of reducing alkaline solutions of certain metallic salts. The reducing action of glucose, lactose and other sugars upon alkaline solutions of copper, silver, mercury, bismuth and other metals has already been mentioned. In the case of silver and glucose, for example, the reaction when carefully controlled proceeds as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 9\,\underset{\text{Silver oxide}}{Ag_2O} = 18\,\underset{\text{Silver}}{Ag} + 3\,\underset{\text{Oxalic acid}}{(COOH)_2} + 3\,\underset{\text{Water.}}{H_2O}.$$

If the weight of reduced silver be determined for this reaction, the amount of glucose can easily be estimated. But unfortunately the reducing action of sugars upon metallic salts does not proceed with the quantitative precision of the above equation; the reduction is rarely complete and the amount of reduced metal varies with the conditions of the experiment. The latter difficulty is obviated, however, in practice by controlling the process so that the same weight of reduced metal is always obtained for the same weight of sugar.

Of the various alkaline solutions of metals those of copper are employed almost exclusively in sugar analysis.

### Copper Reduction Methods

**Early Methods.** — The reducing action of sugars upon different salts of copper has been known since the first beginning of chemistry. Trommer,* in 1841, first noted the value of alkaline copper-sulphate solution as a means of distinguishing grape from cane sugar. Trommer's method was improved in 1844 by Barreswil † who made the important discovery that addition of potassium tartrate to the alkaline copper-sulphate solution greatly increased its stability. Barreswil's method was volumetric; the sugar solution was slowly added to the boiling copper reagent, which had previously been standardized against pure glucose, until the blue color was just discharged.

* Ann., **39,** 360.

† Journal de Pharmacie [3], **6,** 301.

**Fehling's Method.** — Fehling,* in 1848, first worked out the details of the alkaline copper method, as they now stand, and the copper-sulphate and alkaline-tartrate reagent has since been called by his name.

The copper solution employed by Fehling consisted of 40.00 gms. copper sulphate, $CuSO_4.5\,H_2O$, 160 gms. neutral potassium tartrate and 600–700 gms. sodium hydroxide sol. of 1.12 sp. gr. dissolved in water to 1154.4 c.c. This is equivalent to 34.65 gms. $CuSO_4.5\,H_2O$ dissolved to 1000 c.c., the proportion used by nearly all subsequent workers down to the present time.

Fehling's solution contains 8.822 gms. copper to 1000 c.c. or 0.008822 gm. to 1 c.c. According to Fehling's experiments 1 c.c. of his solution was exactly reduced by 0.005 gm. of anhydrous glucose, or 1 part glucose reduced 1.765 parts copper. In terms of the molecular weight of glucose the ratio would be $180 \times 1.765 = 317.6$. Dividing this value by 63.6, the atomic weight of copper, the atoms of copper reduced by one molecule of glucose is found to be five. The reduction ratio 1 : 5 was regarded as constant by Fehling and was so employed by all chemists until Soxhlet † showed in 1878 that the ratio between sugar and amount of copper reduced was not a constant but varied according to the excess of copper which is present during the reaction.

The more modern methods of sugar determination, which employ Fehling's solution, may be divided into two general classes. I. Volumetric methods based upon the complete reduction of a measured volume of standard solution. II. Methods based upon a gravimetric or volumetric determination of the reduced copper.

### VOLUMETRIC METHODS BASED UPON THE COMPLETE REDUCTION OF A MEASURED VOLUME OF FEHLING'S SOLUTION

**Soxhlet's Method.** — Owing to the decomposition which takes place in the mixed copper-sulphate and alkaline-tartrate solution upon standing, the two solutions employed in the Soxhlet and all other modern methods are mixed only just before using. The solutions consist of the following: *Solution A*, 34.639 gms. of pure crystallized $CuSO_4.5\,H_2O$ are dissolved in water and made up to 500 c.c. *Solution B*, 173 gms. of Rochelle salts are dissolved in water, 100 c.c. of a solution of caustic soda, containing 516 gms. NaOH per liter are added, and the volume completed to 500 c.c. Previous to analysis mix equal volumes of solutions *A* and *B*.

Before using the mixed copper reagent, it should be standardized against glucose, invert sugar, lactose, etc., according to the needs of

* Ann., **72**, 106; **106**, 75. † J. prakt. Chem. [2], **21**, 227.

analysis. Since reducing sugar in sugar-cane, sugar-beet and most other food products is most usually expressed as invert sugar, the latter is most commonly used for standardization. A standard solution of invert sugar has also an advantage in being easily prepared.

*Standard Invert Sugar Solution. Method of the Association of Official Agricultural Chemists.** — Dissolve 4.75 gms. of pure sucrose in 75 c.c. of water, add 5 c.c. of 38.8 per cent hydrochloric acid and set aside during a period of 24 hours at a temperature not below 20° C. Neutralize the acid exactly with dilute sodium hydroxide and make up to 1000 c.c.; 100 c.c. of this solution contains 0.500 gm. of invert sugar.

The amount of standard invert sugar solution necessary to reduce 100 c.c. of the mixed copper reagent is determined according to the details described in the next paragraph.

*Application to Analysis of Sugar Products. Method of the Association of Official Agricultural Chemists.†* — Make a preliminary titration to determine the approximate percentage of reducing sugar in the material under examination. Prepare a solution which contains approximately 1 per cent of reducing sugar. Place in a beaker 100 c.c. of the mixed copper reagent and approximately the amount of the sugar solution for its complete reduction. Boil for two minutes. Filter through a folded filter and test a portion of the filtrate for copper by use of acetic acid and potassium ferrocyanide. Repeat the test, varying the volume of sugar solution, until two successive amounts are found which differ by 0.1 c.c., one giving complete reduction and the other leaving a small amount of copper in solution. The mean of these two readings is taken as the volume of the solution required for the complete precipitation of 100 c.c. of the copper reagent.

Under these conditions 100 c.c. of standard copper reagent require 0.475 gm. of anhydrous glucose or 0.494 gm. of invert sugar for complete reduction. Calculate the glucose by the following formula:

$V$ = the volume of the sugar solution required for the complete reduction of 100 c.c. of standard copper reagent.

$W$ = the weight of the sample in 1 c.c. of the sugar solution.

Then $$\frac{100 \times 0.475}{V \times W} = \text{per cent of glucose,}$$

or $$\frac{100 \times 0.494}{V \times W} = \text{per cent of invert sugar.}$$

In making the test for unreduced copper a few drops of the filtered solution are placed upon a white test plate, acidified with a few drops of

* Bull. 107 (revised) U. S. Bur. of Chem., p. 42. † Ibid.

10 per cent acetic acid and a drop of 2 per cent potassium-ferrocyanide solution added. A brown coloration indicates the presence of unreduced copper.

**Volume of Fehling's Solution Reduced by Different Sugars.** — The ratio between volume of standard Fehling's solution and the amount of different sugars, just sufficient to cause complete reduction, was determined by Soxhlet * to be as follows:

TABLE LXX

| Volume of Fehling's solution reduced by different sugars. | | | | | | | | | Reducing power in terms of glucose. |
|---|---|---|---|---|---|---|---|---|---|
| 0.5000 | gm. | glucose | reduces | 105.2 | c.c. | Fehling's | solution | ..... | 1.000 |
| 0.5000 | " | invert sugar | " | 101.2 | " | " | " | ..... | 0.962 |
| 0.5000 | " | fructose | " | 97.2 | " | " | " | ..... | 0.924 |
| 0.5000 | " | lactose | " | 74.0 | " | " | " | ..... | 0.703 |
| 0.5000 | " | maltose | " | 64.2 | " | " | " | ..... | 0.610 |

The above results calculated to equal volumes of copper reagent show that 100 c.c. of mixed standard Fehling's solution are reduced by 0.4753 gm. of glucose, 0.4941 gm. of invert sugar, 0.5144 gm. of fructose, 0.6757 gm. of lactose and 0.7788 gm. of maltose.

**Modifications of Soxhlet's Method.** — Instead of employing 100 c.c. of Fehling's solution for the Soxhlet determination, it is more customary to use 10 c.c., 20 c.c. or 50 c.c., the quantity thus used being measured into a casserole, beaker or flask, and diluted, according to requirements, with a measured volume of water. In case of very dilute sugar solutions, as small a quantity as 5 c.c. of Fehling's solution may be used to advantage.

In using any of the numerous modifications of Soxhlet's method, it is important that the Fehling solution be standardized under exactly the same conditions as in analysis. The same degree of dilution should be followed for the mixed copper reagent in all experiments. Soxhlet found that 0.5 gm. of glucose reduced 105.2 c.c. of Fehling's solution when undiluted and only 101.1 c.c. when diluted with 4 parts of water; similar results were also obtained with other sugars. Such differences as these might produce a variation of several per cent in the estimation of reducing sugars.

It is also evident that to obtain the most concordant results the sugar solutions should always contain about the same percentage of reducing sugar. This is accomplished in practice by making a rough

* J. prakt. Chem. [2] **21**, 227.

preliminary determination and then making up a fresh sugar solution so that the percentage of reducing sugar shall be 0.1 per cent, 0.5 per cent or 1.0 per cent, etc., according to the volume of Fehling's solution taken and the individual preference of the chemist. In this manner approximately the same volume of sugar solution is always used for reducing the same volume of copper reagent, and under such conditions, with a uniform method of boiling, the most accurate results are obtained.

A difference in reducing power is also obtained whether the sugar solution be added to the copper reagent in small portions, with successive periods of boiling, or only in one portion with one period of boiling. The most accurate results are secured where the test is made with the entire volume of sugar solution, necessary for complete reduction, with only one period of boiling.

The following example will give an illustration of the application of the method:

*Example.* — 20 c.c. of Fehling's solution diluted with 80 c.c. of water were found to require for reduction exactly 20.2 c.c. of standard invert sugar solution or 0.101 gm.

50 gms. of sugar-cane molasses were diluted to 1000 c.c. Of this solution about 8 c.c. were required to discharge the blue color of 20 c.c. Fehling's solution.

80 c.c. of the sugar solution (4 gms. molasses) were then made up to 200 c.c. (1 c.c. = 0.02 gm. molasses). Of this solution 19.6 c.c. when boiled with 20 c.c. Fehling's solution and 80 c.c. of water for 2 minutes showed incomplete reduction by the ferrocyanide test and 19.8 c.c. complete reduction.

Calling 19.7 c.c. the volume of sugar solution necessary to reduce the 20 c.c. of Fehling's solution, then $\frac{0.101 \times 100}{0.02 \times 19.7} = 25.64$ per cent invert sugar in the molasses.

**The Ferrocyanide Test for Copper.** — Several methods are followed for making the ferrocyanide test for unreduced copper. It sometimes happens that the cuprous oxide is precipitated in a very finely divided form, and gives annoyance by running through the filter.

One method of making the test is to superimpose several small strips of filter paper and allow a few drops of the solution to fall upon the upper paper. The moistened area upon the second or third underlying strip is then treated with a drop of ferrocyanide solution acidified with acetic acid. The appearance of a brown spot indicates the presence of unreduced copper.

Another method of removing the portion of solution to be tested is by means of a Wiley or Knorr filtering tube, which is prepared as follows:

*Wiley's Filter Tube.* — The Wiley * filter tube, Fig. 166*a*, consists of a piece of glass tubing, 5 to 7 mm. in diameter and 20 to 25 cm. long, one end of which has been softened in a flame and then pressed out so as to form a shoulder. A piece of fine linen is then stretched tightly over the end and tied securely by a thread. In using the tube the covered end is dipped into water containing in suspension finely divided asbestos, and a film of the latter spread over the surface of the filter by suction at the upper end. A small portion of the liquid to be tested is sucked into the tube and then poured from the open end onto the test plate. Knorr's † modification of the Wiley tube is of smaller diameter and contains a perforated platinum disk in place of the linen (Fig. 166*b*). The disk is coated with asbestos and the liquid withdrawn for testing as with the Wiley tube. The filter tubes should not be reused until after cleaning in dilute nitric acid and washing with water.

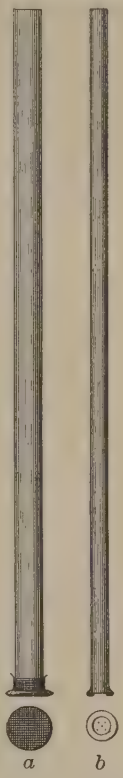



Fig. 166. — Filter tubes for determining reducing sugars.

*Method of Ross.* — A method due to Ross,‡ and employed quite extensively in Louisiana, is to dip the point of a small folded filter, held by means of forceps, below the surface of the hot solution in the casserole and withdraw a few drops of the clear liquid from the interior of the filter by means of a medicine dropper (Fig. 167). The method is simple, and particularly useful where there is a large amount of routine.

Conveniences for making the determination by Soxhlet's method, such as 2-minute sand glass for regulating time of boiling, test plate, dropping bottles for ferrocyanide solution and acetic acid, are shown in Fig. 167.

**Violette's Method.** — The volumetric method of copper reduction, which is used most extensively in France, is that of Violette.§ The proportions of copper sulphate, Rochelle salts and alkali employed in the Soxhlet method may be used in the Violette determination, or the Violette solution may be taken which consists of

* Wiley's "Agricultural Analysis" (1897), III, 130.
† Ibid.
‡ Journal of Analytical Chemistry, **4** (1890), p. 427.
§ Sidersky's "Manuel" (1909), p. 95.

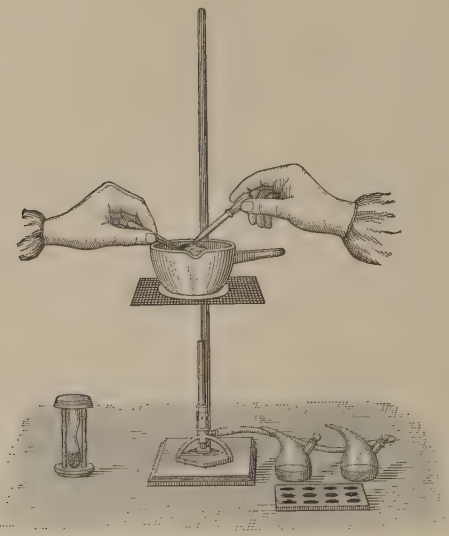


Fig. 167. — Ross's method for determining reducing sugars.

36.46 gms. $CuSO_4.5\ H_2O$, 200 gms. Rochelle salts and 500 gms. sodium hydroxide solution of 1.2 sp. gr. made up to 1000 c.c.

The Violette solution takes a slightly larger amount of copper sulphate than the Soxhlet solution in order that 1 c.c. may correspond to the invert sugar derived from 5 mgs. of sucrose or $\frac{340}{342} \times 5 = 5.263$ mgs. of invert sugar. The ratio of invert sugar and copper sulphate for the Soxhlet and Violette solutions is accordingly 5 : 34.64 :: 5.263 : 36.46.

The Violette solution is preferred by some chemists for convenience in determining sucrose by the method of inversion and copper reduction.

The end point of the reduction in Violette's method is determined, as in the early process of Barreswil, by the disappearance of blue color from the copper solution. The details of the method are as follows:

Ten cubic centimeters of the mixed copper solution are transferred to a large test tube 20 to 22 mm. in diameter and 22 to 24 cm. long; 5 c.c. of distilled water are added in case the solution is rich in reducing sugars and a few small pieces of pumice stone, which have been ignited and then washed in acid and water. The copper solution is then heated to boiling, the grains of pumice stone giving a smooth ebullition and preventing the sudden ejection of liquid from the tube. The sugar

solution to be tested, which should have been previously clarified and diluted to about 0.5 to 1.0 per cent invert sugar, is then added from a burette, a few cubic centimeters at a time, the copper solution being boiled for 2 minutes after each addition. As the reduction proceeds the blue color of the solution becomes more of a reddish violet, due to the diminishing intensity of the blue and the increasing amount of the red cuprous oxide. Towards the end of the reduction it is necessary to hold the tube against a white wall or paper and observe the color of the clear solution, after the red oxide begins to settle. When the final drop of sugar solution discharges the last trace of blue color, the reading of the burette is noted, and the calculation of sugar made as previously described.

A little practice is required in the Violette method in following the disappearance of the blue color. The chemist should standardize his solution against invert sugar, following the same procedure in determining end point as in making an analysis.

The Violette method is much simpler than the Soxhlet method and is for this reason preferred by many chemists. The Soxhlet method, on the other hand, owing to the more sensitive method of determining the end point of reduction, has a much greater degree of accuracy.

The Violette method has been modified by Spencer,* so as to include the ferrocyanide test for unreduced copper. Some chemists have also sought to improve the method by employing larger test tubes and using 20 c.c. of the mixed copper solution. The possibilities of modification in this direction are of course unlimited and do not require special description.

**Pavy's Method.** — Another volumetric process, using the disappearance of blue color as end point, is the method of Pavy,† which is based upon the fact that when Fehling's solution is reduced in presence of ammonia the precipitated cuprous oxide is dissolved as a colorless solution, any unreduced copper being indicated by the characteristic blue color of the cuprammonium compounds. The disturbing influence of the precipitated cuprous oxide upon the color of the solution is thus avoided and, in the absence of air, the change from blue to colorless at the end point becomes quite sharp.

Pavy's copper solution is prepared as follows: 34.65 gms. $CuSO_4.5\,H_2O$, 170 gms. Rochelle salts and 170 gms. potassium hydroxide are dissolved in water to 1000 c.c. It is preferable, however, as in Soxhlet's method to make up the copper and alkali-tartrate solu-

* Spencer's "Handbook for Cane Sugar Manufacturers" (1906), p. 131.

† Pavy's "Physiology of the Carbohydrates" (London, 1894), p. 71.

tions separately to 500 c.c., and to mix equal quantities of the two just before using; 120 c.c. of the mixed copper solution are transferred to a liter flask, 300 c.c. of ammonia of specific gravity 0.880 are added and the volume completed to 1000 c.c.; 20 c.c. of the ammoniacal Fehling's solution are reduced by 0.01 gm. glucose.

The reduction is carried out in a flask of about 150 c.c. capacity, provided with a two-hole stopper, one opening of which is connected with the tip of the burette containing the sugar solution and the other with a bent glass tube for the escape of air and steam (Fig. 168).

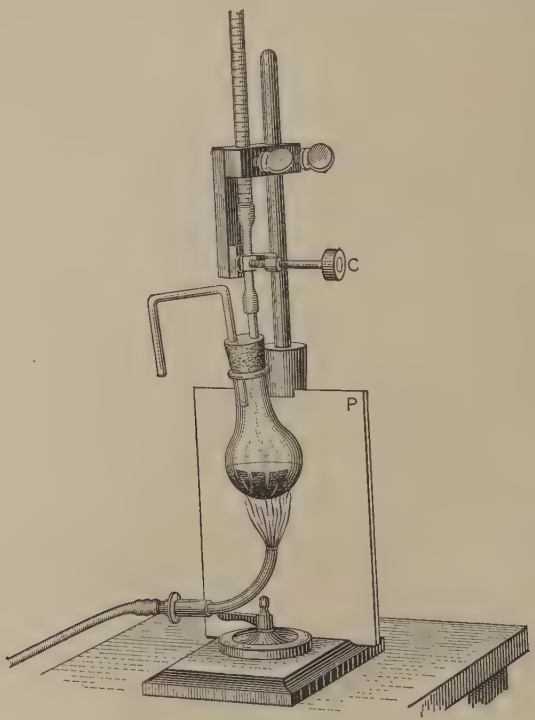



Fig. 168. — Pavy's method for determining reducing sugars.

Forty cubic centimeters of the ammoniacal copper solution are placed in the flask, and after inserting the stopper the solution is brought to a gentle boil. The sugar solution is then added at the rate of 60 to 100 drops per minute, the discharge being regulated by a Pavy pinch cock

(*C*); the ebullition must be maintained without interruption. When the blue color begins to lighten, the sugar solution is added drop by drop until the last trace of color is just discharged. The end point is made more sensitive by looking through the solution against a white plate (*P*).

The reduction must be made in complete absence of air, otherwise the dissolved cuprous oxide will be reoxidized. A precaution sometimes used to prevent the entrance of air, through momentary cooling, is to use a bent-glass exit tube, fitted with a rubber valve, dipping into a beaker of water. Care must also be taken not to prolong the time of reduction, otherwise all the ammonia will be expelled and the cuprous oxide not be dissolved.

In Pavy's method 1 molecule of glucose reduces 6 molecules of cupric oxide instead of 5 molecules as by Fehling's solution. These proportions vary somewhat, however, according to concentration and other conditions of experiment. The solution should, therefore, be standardized against glucose or invert sugar following the exact method employed in analysis.

Pavy's method gives good results, when the reduction is carried out with complete exclusion of the air. The extra precautions necessary for making the determination, and the failure of the method to give good results with colored solutions, have prevented the process from becoming generally employed.

**Conversion Tables for Volumetric Determination of Sugars.** — The calculation of reducing sugars by any of the volumetric methods is much simplified by the use of appropriate conversion tables. If a volume of Fehling's solution be taken, which always corresponds to a fixed amount of reducing sugar, as, for example, 0.5 gm. in Table LXX, and the sugar solution for titration be made up so as to contain this same amount of substance (as 0.5 gm.) in 1 c.c., then the formula for determining reducing sugar becomes

$$R = \frac{0.5 \times 100}{0.5 \times V} = \frac{100}{V},$$

in which $R$ is the per cent of reducing sugar in the substance and $V$ the cubic centimeters of sugar solution necessary for the reduction.

If the substance be very high or very low in reducing sugar, an even fraction or multiple of 0.5 gm. may be taken for the amount of substance to be dissolved in 1 c.c. Thus for 0.05 gm. of substance in 1 c.c. $R = \frac{1000}{V}$, and for 1 gm. of substance in 1 c.c. $R = \frac{50}{V}$.

Under the above conditions of analysis a table giving different multiples of the reciprocals of the burette readings will give the corresponding percentages of reducing sugars. The following example will illustrate the method for constructing such a table.

*Fehling's solution taken* = 0.2 *gram of reducing sugar*

| Volume of sugar solution for reduction. | Reciprocal. | Weight of substance in 1 c.c. of sugar solution. | | | | |
|---|---|---|---|---|---|---|
| | | 0.40 gm. | 0.20 gm. | 0.10 gm. | 0.04 gm. | 0.02 gm. |
| $V$ | $\frac{1}{V}$ | $\frac{50}{V}$ | $\frac{100}{V}$ | $\frac{200}{V}$ | $\frac{500}{V}$ | $\frac{1000}{V}$ |
| c.c. | | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. |
| 20.0 | 0.05000 | 2.50 | 5.00 | 10.00 | 25.00 | 50.00 |
| 20.1 | 0.04975 | 2.49 | 4.98 | 9.95 | 24.88 | 49.75 |
| 20.2 | 0.04950 | 2.48 | 4.95 | 9.90 | 24.75 | 49.50 |
| 20.3 | 0.04926 | 2.46 | 4.93 | 9.85 | 24.63 | 49.26 |
| 20.4 | 0.04902 | 2.45 | 4.90 | 9.80 | 24.51 | 49.02 |
| .... | ..... | .... | .... | .... | ..... | ..... |
| 30.0 | 0.03333 | 1.67 | 3.33 | 6.67 | 16.67 | 33.33 |
| 40.0 | 0.02500 | 1.25 | 2.50 | 5.00 | 12.50 | 25.00 |
| 50.0 | 0.02000 | 1.00 | 2.00 | 4.00 | 10.00 | 20.00 |

The table can of course be modified in a great variety of ways to suit individual requirements. A list of reciprocals for assistance in calculating such a table is given in the Appendix (Table 25).

**Reischauer and Kruis's Method.** — In the methods previously described a constant volume of Fehling's solution was taken and the amount of sugar solution noted necessary to complete the reduction. In a process first proposed by Lippmann * and elaborated by Reischauer and Kruis † the opposite procedure is followed. A constant volume of sugar solution is taken and the amount of Fehling's solution determined necessary to oxidize the reducing sugar.

In the Reischauer-Kruis method the sugar solution is made up so as not to contain over 0.58 gm. glucose in 100 c.c. Six numbered test tubes holding from 20 to 30 c.c. are taken and 5 c.c. of the sugar solution measured into each; 1, 2, 3, 4, 5 and 6 c.c. respectively of Fehling's solution are then added to the different tubes, which are afterwards shaken and immersed in boiling water for 20 minutes. At the end of this time the tubes are examined and the two tubes noted in which reduction is just completed and in which the least amount of unreduced copper is left. Having noted the limits between which the true copper equivalent lies, the volume of Fehling's solution is varied

* Oester. Ungar. Z. Zuckerind., **7,** 256.

† Oester. Ungar. Z. Zuckerind., **12,** 254.

within this interval until the exact amount necessary for oxidizing all the reducing sugar is found.

The pipettes employed for this method are graduated in their lower part from 1 c.c. to 5 c.c. and in the stem contain an extra 1 c.c. graduated into hundredths. With three trials and employment of the ferrocyanide test, the volume of Fehling's solution can be determined to 0.01 c.c. The following example illustrates the application of the method.

| First trial. | Second trial. | Third trial. |
|---|---|---|
| 1 c.c. Cu all reduced | 4.15 c.c. Cu all reduced | 4.32 c.c. Cu all reduced |
| 2 " " | 4.30 " " | 4.34 " " |
| 3 " " | 4.45 " Cu in solution | 4.36 " " |
| 4 " " | 4.60 " " | 4.38 " Cu in solution |
| 5 " Cu in solution | 4.75 " " | 4.40 " " |
| 6 " " | 4.90 " " | 4.42 " " |

The quantity of Fehling's solution which exactly oxidizes the reducing sugar in the 5 c.c. of solution may, therefore, be placed at 4.37 c.c.

The amount of glucose corresponding to each 0.01 c.c. between 1 c.c. and 6 c.c. of Fehling's solution is found from a table calculated by Kruis (Appendix, Table 9).

The Reischauer-Kruis method possesses certain advantages over the methods previously described in point of exactness; the error due to variation in reducing power with changes in concentration is avoided, the amount of reducing sugar in 5 c.c. corresponding to different volumes of Fehling's solution being definitely known for the conditions of experiment. The large amount of labor and time necessary for completing a determination has been, however, a serious obstacle against the general use of the method.

### METHODS BASED UPON A GRAVIMETRIC OR VOLUMETRIC DETERMINATION OF REDUCED COPPER

In the methods of this class an excess of copper is present in the Fehling's solution at the end of reduction. The precipitated cuprous oxide after a fixed period of heating is filtered off, and the amount of copper determined by any of the numerous gravimetric or volumetric processes. The weight of reducing sugar corresponding to a definite weight of precipitated copper is then determined by means of formulæ or tables which have been calculated from results obtained upon known amounts of pure sugar under similar conditions of experiment.

**Variability in Reducing Power of Monosaccharides.** — Soxhlet* showed that when a solution of glucose acted upon Fehling's solution the first portion added reduced most strongly and the succeeding portions gradually less so. This variability in reducing power is found to be different, however, for the monosaccharides, glucose, fructose, invert sugar, galactose, etc., than for the disaccharides, lactose and maltose.

As examples of the variability in reducing power of monosaccharides the following results are given. The values, which were calculated from Bertrand's sugar tables, represent the milligrams of copper reduced by each succeeding 10-milligram portion of added sugar.

TABLE LXXI

*Showing variability in reducing power of monosaccharides*

| Number of series. | Invert sugar. Milligrams copper. | Glucose. Milligrams copper. | Galactose. Milligrams copper. |
|---|---|---|---|
| First 10 mgs. of sugar reduce..... | 20.6 | 20.4 | 19.3 |
| Second 10 " " " ..... | 19.8 | 19.7 | 18.6 |
| Third 10 " " " ..... | 18.9 | 19.0 | 18.3 |
| Fourth 10 " " " ..... | 18.4 | 18.4 | 17.7 |
| Fifth 10 " " " ..... | 17.7 | 17.9 | 17.3 |
| Sixth 10 " " " ..... | 17.2 | 17.4 | 16.9 |
| Seventh 10 " " " ..... | 16.6 | 17.0 | 16.7 |
| Eighth 10 " " " ..... | 16.1 | 16.3 | 16.3 |
| Ninth 10 " " " ..... | 15.8 | 15.9 | 16.3 |
| Tenth 10 " " " ..... | 15.4 | 15.8 | 16.0 |

It is seen that each succeeding 10 mgs. of added glucose undergoes a loss in reducing power of about 3 per cent.

**Law of Reducing Action.** — The reducing action of a monosaccharide upon Fehling's solution may be expressed in general terms as follows:

If for the first minute quantity $s$ of a given sugar a definite amount $c$ of copper is reduced, then for any multiple $n$ of $s$ the weight of copper would be $nc$, if the same amount of copper in the Fehling's solution were always maintained. The latter condition, however, is never realized in practice, and with the continuous removal of copper from solution the value $nc$ becomes $nc - (n-1+n-2+n-3+\cdots n-n)k$. When working with weighable quantities of sugar, this expression should be modified to $c+(n-1)d-(n-2+n-3+\cdots n-n)k$ in which $d$ is the difference between the weights of copper for the first two members of the series $s$ and $2s$. The values of $d$ and of the constant

* J. prakt. Chem. [2], **21**, 227.

$k$ are easily determined empirically, and knowing these it is possible to construct tables for any of the reducing sugars.

As an example of this method of calculation the following values are taken from the experimental work of Allihn: *

No. of series ($n$).

1 . . . . . 10 mgs. of glucose reduce 18.0 mgs. copper
2 . . . . . 20 mgs. of glucose reduce 38.2 mgs. copper
25 . . . . . 250 mgs. of glucose reduce 463.0 mgs. copper.

$$18.0 = c.$$

$$38.2 - 18.0 = 20.2 = d.$$

Substituting the above values for $c$ and $d$ in the equation for $n = 25$,

$$18 + (25 - 1)\,20.2 - (25 - 2 + 25 - 3 \ldots)\,k = 463.0$$

whence $k = 0.14$.

The equation $18 + (n - 1)\,20.2 - (n - 2 + n - 3 + \cdots n - n)\,0.14$ will give the milligrams of copper reduced by any multiple $n$ of 10 mgs. of glucose under the conditions of Allihn's experiments.

Suppose it is required to find the milligrams of copper reduced by 100 mgs. of glucose.

$$18 + (10 - 1)\,20.2 - (10 - 2 + 10 - 3 \ldots)\,0.14 = 194.8 \text{ mgs. Cu.}$$

Allihn obtained by actual experiment 195 mgs. of copper by the reducing action of 100 mgs. of glucose.

**Calculation of Reduction Tables.** — The calculation of tables for the copper-reducing power of different sugars is usually made by the method of least squares, according to the general formula:

$$y = A + Bx + Cx^2,$$

in which $x$ is the milligrams of copper reduced by $y$ milligrams of sugar and $A$, $B$ and $C$ constants. Having determined by experiment the values of $x$ for 10 or more values of $y$, the calculation of $A$, $B$ and $C$ is made in the same manner as described on page 165.

As an example of the method of least squares the work of Allihn is again quoted. Allihn found that different amounts of glucose under constant conditions of experiment reduced the following amounts of copper.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mgs. of glucose ($y$) . . . | 10.0 | 20.0 | 25.0 | 50.0 | 100.0 | 125.0 | 150.0 | 175.0 | 200.0 | 225.0 | 250.0 |
| Mgs. of copper ($x$) . . . . | 18.0 | 38.2 | 47.5 | 99.0 | 195.0 | 242.5 | 287.7 | 333.0 | 377.7 | 421.2 | 463.0 |

Substitution of the above values for $x$ and $y$ in the formula $y = A + Bx + Cx^2$ gives the general equation

$$y = -2.5647 + 2.0522\,x - 0.0007576\,x^2,$$

by means of which Allihn constructed his table giving the milligrams of glucose corresponding to any weight of reduced copper between 10 mgs. and 463 mgs.

* J. prakt. Chem. [2], **22**, 46.

**Variability in Reducing Power of Disaccharides.** — The variability in reducing power of maltose and lactose is different from that noted for the monosaccharides. According to the amount of free alkali, time of boiling and other conditions, succeeding portions of maltose and lactose, while usually showing a slight loss, may show either no change at all, or even a slight gain in reducing power over preceding portions of the same sugar. This peculiarity of maltose and lactose is explained by a slight hydrolysis of the sugar into monosaccharides of higher reducing power. A slight inversion of this kind takes place with sucrose, which is strictly speaking a non-reducing sugar, and it no doubt occurs to a greater or less extent with all higher saccharides upon boiling with Fehling's solution.

As an illustration of the reducing power of successive portions of maltose, the following results are taken from the tables of Wein and of Munson and Walker.

TABLE LXXII

*Showing variability in reducing power of maltose*

| Number of Series. | Wein. | Munson and Walker. |
|---|---|---|
| | Mgs. Cu. | Mgs. Cu. |
| First 30 mgs. of maltose reduce..... | 35.4 | 35.9 |
| Second 30 " " " ..... | 34.5 | 33.6 |
| Third 30 " " " ..... | 34.0 | 33.5 |
| Fourth 30 " " " ..... | 33.4 | 33.8 |
| Fifth 30 " " " ..... | 33.4 | 33.6 |
| Sixth 30 " " " ..... | 33.8 | 33.7 |
| Seventh 30 " " " ..... | 33.5 | 33.6 |

It is seen that in both series of experiments there is at first a marked decrease and then a slight increase in the reducing power of the successive portions of added sugar. Changes of a similar nature are noted in some of the tables for lactose.

The reducing power of the disaccharides upon Fehling's solution is much more subject to change with difference in conditions than the monosaccharides. Kjeldahl,* for example, found that increasing the amount of alkali in Fehling's solution caused the reducing power of maltose and lactose to gain with ten times the rate of increase noted for glucose. The same effect is also produced by prolonging the time of boiling. This greater sensibility of the disaccharides to disturbing influences during reduction involves a greater experimental error in the determination when the details of the method are not carefully followed.

* Neue Z. Rübenzuckerind., **37**, 13, 23.

Methods and tables for estimating different sugars from the amount of copper reduced from Fehling's solution have been devised by Soxhlet; Allihn; Wein; Meissl; Herzfeld; Lehmann; Kjeldahl; Pflüger; Ost; Hönig and Jesser; Brown, Morris and Millar; Bertrand; Defren; Munson and Walker; Kendall; and many others. It is impossible to describe all these processes and only a few of the more typical methods will be selected. The method of Allihn,* which is one of the widest known, illustrates well the various principles involved and will be described first in somewhat fuller detail.

**Allihn's Method for the Determination of Glucose.** — The following details of Allihn's method with the description of several processes for determining the amount of reduced copper are taken from the Methods of Analysis of the Association of Official Agricultural Chemists.†

PREPARATION OF REAGENTS

*Copper-sulphate Solution.* — Dissolve 34.639 gms. of $CuSO_4.5H_2O$ in water and dilute to 500 c.c.

*Alkaline-tartrate Solution.* — Dissolve 173 gms. of Rochelle salts and 125 gms. of potassium hydroxide in water and dilute to 500 c.c.

DESCRIPTION OF METHOD

Place 30 c.c. of the copper solution, 30 c.c. of the alkaline-tartrate solution and 60 c.c. of water in a beaker and heat to boiling. Add 25 c.c. of the solution of the material to be examined, which must be so prepared as not to contain more than 0.250 gm. of glucose, and boil for exactly two minutes keeping the beaker covered. Filter immediately through asbestos without diluting, and obtain the weight of copper by one of the methods described in the following section. The corresponding weight of glucose is found from Allihn's table (Appendix, Table 10).

METHODS FOR DETERMINING REDUCED COPPER

**Reduction of the Cuprous Oxide in Hydrogen.**‡ — "Filter the cuprous oxide immediately through a weighed filtering tube made of hard glass, using suction. Support the asbestos film in the filtering tube with a perforated disk or cone of platinum, and wash free from loose fibers before weighing; moisten previous to the filtration. Provide the tube with a detachable funnel during filtration, so that none of the precipitate accumulates near the top, where it could be removed by

* J. prakt. Chem. [2], **22,** 46.

† Bull. 107 (revised), U. S. Bur. of Chem., pp. 49–53.

‡ Ibid.

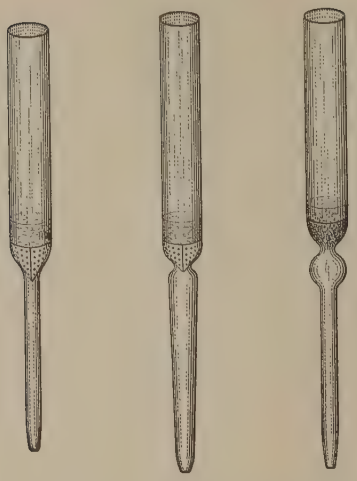



Fig. 169. — Forms of tubes for filtering cuprous oxide.

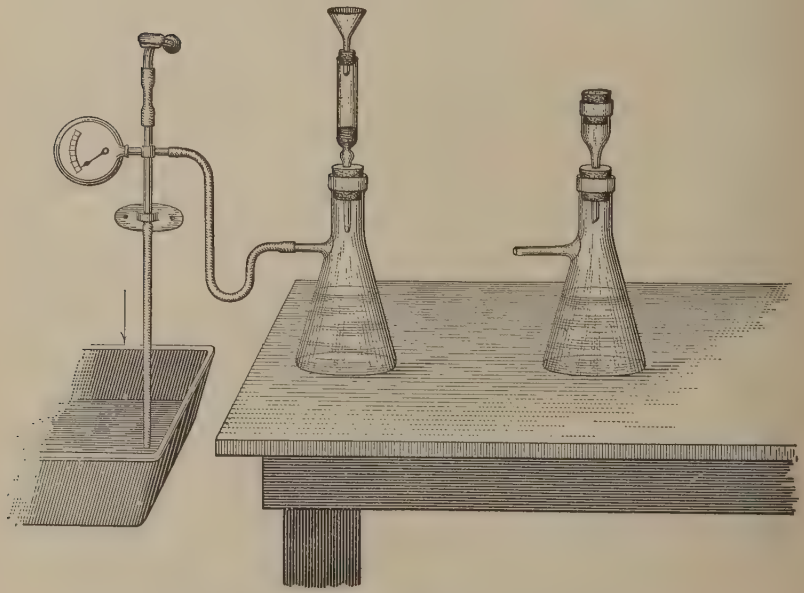


Fig. 170. — Showing methods of filtering cuprous oxide with filter tube or Gooch crucible.

the cork used during the reduction of the cuprous oxide. Transfer all the precipitate to the filter and thoroughly wash with hot water, following the water by alcohol and ether successively. After being dried, connect the tube with an apparatus for supplying a continuous current of dry hydrogen, gently heat until the cuprous oxide is completely reduced to the metallic state, cool in the current of hydrogen and weigh."

Several forms of tubes for filtering cuprous oxide are shown in Fig. 169. Glass wool is sometimes used in place of a platinum disk for holding the asbestos, but makes a less resistant support (see Fig. 169 III).

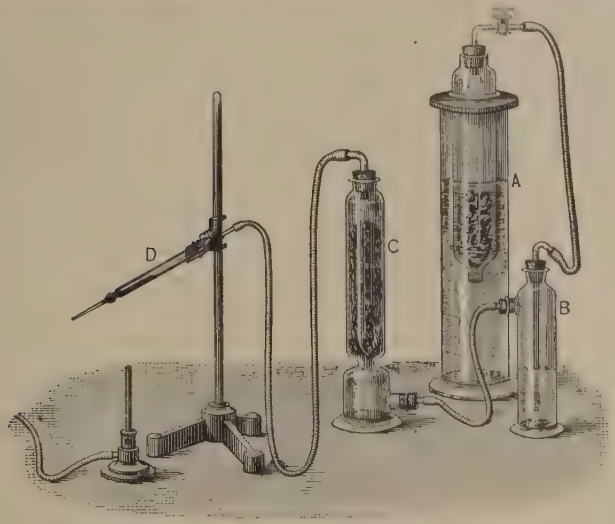



Fig. 171. — Apparatus for reducing cuprous oxide to copper. *A*, hydrogen generator; *B* and *C*, gas driers; *D*, filter tube containing cuprous oxide.

A convenient method of filtering cuprous oxide by means of suction is shown in Fig. 170. A continuous filtration should be maintained and all the precipitate should be transferred to the tube before the liquid above the asbestos is allowed to run completely through. Too rapid or too irregular filtration may cause particles of cuprous oxide to run through the asbestos. A fine jet of water will usually bring all the cuprous oxide into the filter tube; should any of the precipitate remain adhering to the beaker a feather, or a rubber-tipped rod, will assist the removed.

The reduction of the cuprous oxide to copper by means of hydrogen is shown in Fig. 171. All air must be expelled from the tube before

heating, otherwise there is danger of explosion. The heating should be continued until all water is expelled from the tube. A desiccator of the form shown in Fig. 172 is convenient for holding filter tubes before weighing.

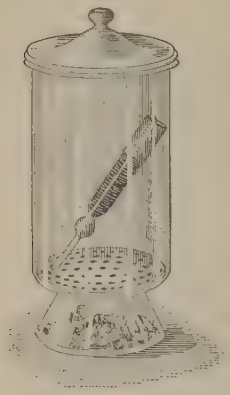


Fig. 172. — Desiccator for filter tubes.

The asbestos used for loading the filter tubes should be of a kind which is not attacked by hot Fehling's solution. The following method of preparation used by Munson and Walker * is recommended.

*Preparation of Asbestos.* — Prepare the asbestos which should be the amphibole variety by first digesting with 1:3 hydrochloric acid for two or three days. Wash free from acid and digest for a similar period with soda solution, after which treat for a few hours with hot alkaline copper-tartrate solution of the strength employed in sugar determinations. Then wash the asbestos free from alkali, finally digest with nitric acid for several hours, and after washing free from acid shake with water for use. In preparing filter tubes or Gooch crucibles load with a film of asbestos one-fourth inch thick, wash this thoroughly with water to remove fine particles of asbestos; finally wash with alcohol and ether, dry for 30 minutes at 100° C., cool in a desiccator and weigh. It is best to dissolve the copper with nitric acid each time after weighing and use the same felts over and over again, as they improve with use.

The method of estimating copper by reduction of the precipitated cuprous oxide, although not so exact as the electrolytic method, is nevertheless sufficiently accurate for most purposes of analysis. In the case of impure sugar products the cuprous oxide is frequently contaminated with mineral or organic matter, and in such cases the method gives too high results.

**Determination of Reduced Copper by Electrolysis.** — *Deposition from Sulphuric-acid Solution.*† — Filter the cuprous oxide in a Gooch crucible (as shown in Fig. 170), wash the beaker and precipitate thoroughly with hot water without any effort to transfer the precipitate to the filter. Wash the asbestos film and the adhering cuprous oxide into the beaker by means of hot dilute nitric acid. After the copper is all in solution, refilter through a thin film of asbestos and wash thoroughly with hot

* J. Am. Chem. Soc., **28**, 666.

† Bull. 107 (revised), U. S. Bur. of Chem., pp. 49–53.

water. Add 10 c.c. of dilute sulphuric acid, containing 200 c.c. of sulphuric acid (sp. gr. 1.84) per liter, and evaporate the filtrate on the steam bath until the copper salt has largely crystallized. Heat carefully on a hot plate or over a piece of asbestos board until the evolution of white fumes shows that the excess of nitric acid is removed. Add from 8 to 10 drops of nitric acid (sp. gr. 1.42) and rinse into a platinum dish of from 100 to 125 c.c. capacity. Precipitate the copper by electrolysis. Wash thoroughly with water, alcohol and ether successively, dry at about 50° C. and weigh. If preferred the electrolysis can be conducted in a beaker, the copper being deposited upon a weighed platinum cylinder.

*Deposition from Sulphuric- and Nitric-acid Solution.** — Filter and wash as previously described. Transfer the asbestos film from the crucible to the beaker by means of a glass rod and rinse the crucible with about 30 c.c. of a boiling mixture of dilute sulphuric and nitric acids, containing 65 c.c. of sulphuric acid (sp. gr. 1.84) and 50 c.c. of nitric acid (sp. gr. 1.42) per liter. Heat and agitate until solution is completed; filter and electrolyze.

*Deposition from Nitric-acid Solution.*† — Filter and wash as previously described. Transfer the asbestos film and adhering oxide to the beaker. Dissolve the oxide still remaining in the crucible by means of 2 c.c. of nitric acid (sp. gr. 1.42), adding it with a pipette and receiving the solution in the beaker containing the asbestos film. Rinse the contents of the beaker until the copper is all in solution, filter, dilute the filtrate to a volume of 100 c.c. or more and electrolyze. When a nitrate solution is electrolyzed, the first washing of the deposit should be made with water acidulated with sulphuric acid in order that the nitric acid may all be removed before the current is interrupted.

**Leach's Electrolytic Apparatus.** — A convenient apparatus for the electrolytic deposition of copper in sugar analysis is that of Leach ‡ shown in Fig. 173. *A* is a hard rubber plate 50 cm. long and 25 cm. wide provided with four insulated metal binding posts *B*, each carrying at the top a thumb screw by which a coiled-platinum-wire electrode may be attached. In front of each post is a copper plate about 4 cm. square covered with thin platinum foil *P*, which is bent around the edges of the copper plate and so held in place, the copper plate being screwed to the rubber from beneath. On the square platinum-covered plate is set the platinum evaporating dish which holds the solution

* Bull. 107 (revised), U. S. Bur. of Chem., pp. 49–53.

† Ibid.

‡ Leach's "Food Inspection and Analysis" (1911), p. 608.

from which the copper is to be deposited, the inside of the dish forming the cathode, while the coiled platinum wire, dipping below the surface of the solution, forms the anode. In front of each platinum-covered plate is a switch *S*, and at either end of the hard-rubber plate is a binding post *R*, for connection with the electric current.

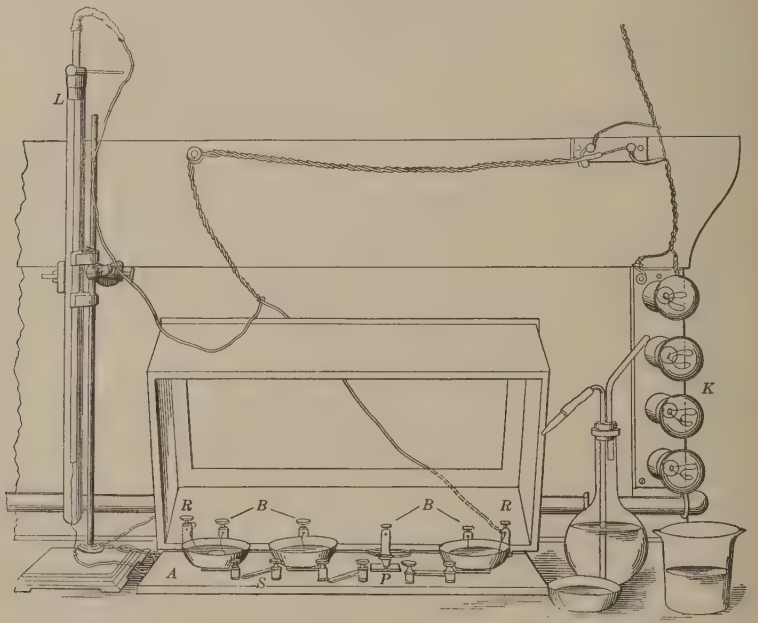



Fig. 173. — Leach's electrolytic apparatus for determining reduced copper.

Four determinations may be carried on simultaneously in four platinum dishes, if desired, the wiring and the switches being so arranged that beginning at one end of the plate either the first dish, or the first two or the first three, may be thrown in or out of the circuit at will without interrupting the current through the remaining dishes. A cover with wooden sides and glass top fits closely over the whole apparatus as a protection from dust, but may easily be lifted off to manipulate the dishes when desired. The sides of the cover are perforated to permit the escape of the gas formed during the electrolysis.

The ordinary street current is used when available, and the strength of the current may be varied within wide limits by means of a number of 16- or 32-candle-power lamps *K*, coupled in multiple, and a rheostat

*L*, consisting of a vertical glass tube sealed at the bottom, containing a column of dilute acid, the resistance being changed by varying the length of the acid column contained between the two platinum terminals immersed therein, one of which is movable. A gravity battery of four cells may be employed if the laboratory is not equipped with electric lights.

In using the apparatus the plating process should go on till all the copper is deposited, which requires several hours or over night with a current of about 0.25 ampere. Before stopping the process the absence of copper in the solution should be proved by removing a few drops with a pipette, adding first ammonia, then acetic acid and testing with ferrocyanide of potassium. If no brown coloration is produced, all the copper has been plated out. Throw the dish out of circuit by means of the switch, pour out the acid solution quickly before it has a chance to dissolve any of the copper, wash the dish first with water and then with alcohol, dry and weigh.

The copper may be removed from the platinum dish by strong nitric acid.

The electrolytic process for determining reduced copper is the most exact of all methods. The determination, however, involves a considerable expenditure of time and for this reason is but little used in sugar laboratories where there is a large amount of routine.

*Electrolytic Method of Peters.* — Peters * has devised a rapid electrolytic method for the determination of copper, whereby the metal is deposited from an alkaline-tartrate solution, such as is used in preparing Fehling's solution. The electrolysis is carried out either in platinum dishes placed upon plates of sheet brass to which the cathode connection is made, or in glass beakers or large test tubes, in which case large cylindrical strips of sheet copper may be used for the cathode. The anode consists of a flat or cylindrical spiral of platinum wire, which should be placed at a distance of 1 cm. or less from the cathode surface. A volume of 10 c.c. copper solution (which may be slightly acid or alkaline) is usually taken, to which is added an approximately equal volume of a solution containing 35 gms. pure Rochelle salts and 25 gms. potassium hydroxide (purified by alcohol) in 100 c.c. For copper solutions containing free sulphuric or nitric acid, two volumes of the alkaline-tartrate solution may be used. From 0.4 to 1.0 c.c. of a saturated aqueous potassium-cyanide solution is then added according to the amount of copper present; the amount of cyanide solution should be less than sufficient to dis-

* J. Am. Chem. Soc., **34**, 426.

charge the blue color. If the copper deposit should be found to be too soft or dark colored, more cyanide should be used; an excess of the latter, however, greatly lengthens the time for complete deposition of the copper.

In making the determination the direct 110-volt current of a lighting system is used with three 32-candle-power lamps interposed as resistance; under these conditions the voltage measures 2.6 and the amperage 2.85. During the electrolysis the solution is warmed by a small burner placed under the brass plate to one side of the cathode vessel; if test tubes are used they are placed upon wire gauze over a small flame. The evolution of gas and the circulation of warm liquid cause a very rapid deposition of copper, which is usually complete in less than 30 minutes. The solution should be covered during electrolysis to prevent loss by spraying.

To determine the completion of electrolysis, Peters recommends the Endemann-Prochazka* hydrobromic acid test. One volume of concentrated sulphuric acid is diluted with 2 to 3 volumes of distilled water. About 1 c.c. of the dilute acid is placed in a narrow test tube, a few crystals of potassium bromide added and the whole heated to boiling. A drop of the solution to be tested is then added; as small an amount as 0.007 mg. copper will cause a red color to develop.

If the deposition of copper is complete, the solution in the cathode vessel, without breaking the current, is displaced by a small stream of water until the resistance lamps are extinguished; under this procedure no copper is lost by solution. The electrode containing the deposit of copper is then washed in alcohol and ether, dried and weighed.

On account of the similarity in composition of the electrolyte employed by Peters to that of the alkaline-tartrate solution used in Allihn's method, the process recommends itself for the determination of copper in the original Fehling's solution or in the filtrate from the reduced cuprous oxide obtained in the analysis of sugar solutions.

Several volumetric processes have been devised for determining copper in the precipitate of cuprous oxide. Of these the permanganate, the iodide and thiocyanate methods will be described.

**Volumetric Permanganate Method.**† — Filter and wash the cuprous oxide as in the previous methods. Transfer the asbestos film to the beaker, add about 30 c.c. of hot water and heat the precipitate and asbestos thoroughly. Rinse the crucible with 50 c.c. of a hot saturated

* Chem. News, **42**, 8.

† Bull. 107 (revised), U. S. Bur. of Chem., pp. 49–53.

solution of ferric sulphate in 20 per cent sulphuric acid, receiving the rinsings in the beaker containing the precipitate. After the cuprous oxide is dissolved, wash the solution into a large Erlenmeyer flask and immediately titrate with a standard solution of potassium permanganate. One cubic centimeter of the permanganate solution should equal 0.010 gm. of copper. In order to standardize the permanganate solution, make six or more determinations with the same sugar solution, titrating one-half of the precipitations and determining the copper in the others by electrolysis. The average weight of copper obtained by electrolysis, divided by the average number of cubic centimeters of permanganate solution required for the titration, is equal to the weight of copper equivalent to 1 c.c. of the standard permanganate solution.

The reaction between the ferric sulphate and cuprous oxide is expressed as follows:

$$Fe_2(SO_4)_3 + Cu_2O + H_2SO_4 = 2\,FeSO_4 + 2\,CuSO_4 + H_2O.$$

Since 1 atom, or 16 parts, of O is required to oxidize the iron reduced by 2 atoms, or 127.2 parts, of Cu, and 1 c.c. of $n/10$ permanganate contains 0.0008 gm. of active O, then 1 c.c. of $n/10$ permanganate is equivalent to 0.00636 gm. Cu. For a solution containing 5 gms. of potassium permanganate to the liter, 1 c.c. will be equivalent very closely to 0.01 gm. of copper. Owing to slight deviations in practice from the above theoretical equation, the copper value of the permanganate must always be determined by direct experiment.

**Volumetric Iodide Method**,* *Low's Modification*.†—*Standardization of the Thiosulphate Solution.*—Prepare a solution of sodium thiosulphate containing 19 gms. of pure crystals to 1000 c.c. Weigh accurately about 0.2 gm. of pure copper foil and place in a flask of 250 c.c. capacity. Dissolve by warming with 5 c.c. of a mixture of equal volumes of strong nitric acid and water. Dilute to 50 c.c., boil to expel the red fumes, add 5 c.c. strong bromine water and boil until the bromine is thoroughly expelled. Remove from the heat and add a slight excess of strong ammonium hydroxide (about 7 c.c. of 0.90 sp. gr.). Again boil until the excess of ammonia is expelled, as shown by a change of color of the liquid, and a partial precipitation. Now add a slight excess of strong acetic acid (3 or 4 c.c. of 80 per cent acid) and boil again for a minute to redissolve the copper. Cool to room temperature and add 10 c.c. of a solution of pure potassium iodide containing 300 gms.

* For a critical study of the iodide method for determining copper in sugar analysis see paper by Peters, J. Am. Chem. Soc., **34,** 422.

† J. Am. Chem. Soc., **24,** 1082.

of potassium iodide to 1000 c.c. Titrate at once with the thiosulphate solution until the brown tinge has become weak, then add sufficient starch liquor to produce a marked blue coloration. Continue the titration cautiously until the color due to free iodine has entirely vanished. The blue color changes toward the end to a faint lilac. If at this point the thiosulphate be added drop by drop and a little time be allowed for complete reaction after each addition there is no difficulty in determining the end point within a single drop. One cubic centimeter of the thiosulphate solution will be found to correspond to about 0.005 gm. of copper.

*Determination of Copper.* — After washing the precipitated cuprous oxide, cover the Gooch crucible with a watch glass and dissolve the oxide by means of 5 c.c. of warm nitric acid (1 : 1), poured under the watch glass with a pipette. Catch the filtrate in a flask of 250 c.c. capacity, wash watch glass and crucible free of copper; 50 c.c. of water will be sufficient. Boil to expel red fumes, add 5 c.c. of bromine water, boil off the bromine and proceed exactly as in standardizing the thiosulphate.

In a later modification of the above method, Low has found it possible to dispense with the use of bromine, the nitrous acid being expelled from the copper solution by boiling, adding ammonia, heating, acidifying with acetic acid and again boiling.

The reaction between the copper acetate and potassium iodide is expressed as follows:

$$2\,Cu(C_2H_3O_2)_2 + 4\,KI = Cu_2I_2 + 4\,KC_2H_3O_2 + I_2.$$

Since 1 atom, or 63.57 parts, of copper liberates 1 atom, or 126.92 parts, of iodine and 1 c.c. of $n/10$ thiosulphate solution (24.8 gms. $Na_2S_2O_3 + 5\,H_2O$ to 1000 c.c.) reacts with 0.01269 gm. I,then 1 c.c. $n/10$ thiosulphate corresponds to 0.00636 gm. Cu. For a solution containing 19.5 gms. of pure sodium thiosulphate to the liter, 1 c.c. will be equivalent very closely to 0.005 gm. of copper. In actual practice the above reaction does not proceed with absolutely quantitative precision, the results of the determination varying somewhat according to concentration of acid, excess of reagents, temperature and other conditions. It is, therefore, important always to standardize the thiosulphate solution against pure copper under the exact conditions which are followed in analysis.

*Kendall's Modification of the Iodide Method.* — The removal of the nitrous acid, formed in dissolving the copper, is the chief difficulty in the iodide method. Kendall * has modified the method by removing

* J. Am. Chem. Soc., **33**, 1947.

the nitrous acid with hypochlorite, the free chlorine, which is evolved, being afterwards removed with phenol.

The cuprous oxide, after filtering and washing upon a Gooch crucible, is dissolved in 10 to 15 c.c. of 30 per cent nitric acid into a 300 c.c. Erlenmeyer flask. The volume of solution and washings should be between 50 and 60 c.c. with an acidity of 4 to 5 c.c. concentrated nitric acid; 5 c.c. of sodium hypochlorite solution are then added of such strength that the iodine liberated by 5 c.c. is equivalent to 30 c.c. of $n/10$ thiosulphate. The solution is allowed to stand 2 minutes, when 10 c.c. of a 5 per cent colorless phenol solution are quickly added. The chlorine gas above the liquid is removed by blowing into the flask and the sides are washed down with a jet of water. The solution is then made slightly alkaline with sodium hydroxide and acidified with acetic acid; 10 c.c. of 30 per cent potassium iodide solution are then added and the free iodine titrated with standard sodium thiosulphate, as under Low's modification, using soluble starch as indicator. The thiosulphate is previously standardized against pure copper under the same conditions as those of the method.

In working with known weights of copper between 20 and 340 mgs., Kendall found the error of his method to exceed in no case 0.3 mg.

*Peters's Modifications of the Iodide Method.*—Peters* has found that boiling the nitric-acid solution of copper in the presence of talcum powder will remove completely all lower oxides of nitrogen and leave the solution, after cooling and diluting, in suitable condition for titration. The copper, or its compound, is dissolved in an Erlenmeyer flask in the least possible volume of concentrated nitric acid, to which one-half its volume of water has been added; 5 to 10 c.c. of dilute acid are sufficient for 0.5 gm., or less, of copper. After solution 15 to 25 c.c. of distilled water and a little pure powdered talcum are added, and the mixture boiled vigorously for 5 to 10 minutes. After cooling to room temperature distilled water is added and 10 c.c. of a saturated potassium-iodide solution, the dilution being so regulated that the final volume of liquid at the end of the thiosulphate titration is about 120 c.c.

Peters has also employed the iodide method in the determination of copper in the alkaline-tartrate solutions, or filtrates, occurring in sugar analysis. In the modification employed, 20 c.c. of Allihn's alkaline-tartrate solution, 20 c.c. of Fehling's copper-sulphate solution and 20 c.c. of water (as in a blank determination), or of the aqueous reducing-sugar solution, were taken, making the total volume for

* J. Am. Chem. Soc., **34**, 422.

reduction always 60 c.c. After the reduction the cuprous oxide is filtered, washed and the filtrate, which has a volume of 70 to 75 c.c., acidified with 4 to 5 c.c. of concentrated sulphuric acid. After cooling to about 20° C., 10 c.c. of saturated potassium iodide are added and the solution titrated with standard thiosulphate in the usual way.

The end point of the titration in the iodide method is best determined according to Peters by noting the point at which a drop of the thiosulphate solution ceases to produce a perceptible white area upon the quiet surface of the titration liquid. As in the case of all other modifications of the iodide method, the thiosulphate solution must be standardized against pure copper under the exact conditions of the analysis.

Potassium iodide is an expensive reagent and where many determinations of copper are made by this method, the waste titration liquids and cuprous iodide precipitates should be saved for recovery of the iodine.

**Volumetric Thiocyanate Method** (Volhard-Pflüger).* — The following solutions are required: (*a*) $n/10$ silver-nitrate solution, (*b*) $n/10$ ammonium-thiocyanate solution, (*c*) a cold saturated solution of sulphur dioxide ($SO_2$) in water, (*d*) nitric acid of sp. gr. 1.2, free from nitrous acid, (*e*) a saturated solution of ferric alum, (*f*) normal sulphuric-acid solution.

The filter tube, or Gooch crucible, containing the cuprous oxide is weighed and the approximate amount of copper determined. The cuprous oxide is then dissolved from the asbestos with nitric acid, the solution treated with a slight excess of normal sulphuric acid solution (*f*) necessary to convert all the copper into copper sulphate and evaporated to dryness. The copper sulphate is then dissolved in water and washed into a 300-c.c. graduated flask. Sodium carbonate solution is added to the point of turbidity and then 50 c.c. of the sulphurous acid reagent (*c*). The solution is boiled for 1 minute and then $n/10$ thiocyanate (*b*) added until there is an excess of about 5 c.c. above the calculated amount necessary for precipitating the copper as cuprous thiocyanate $Cu_2(SCN)_2$. The solution is then cooled, made up to 300 c.c., shaken and filtered through dry filter paper. Should the first runnings appear turbid, they are returned to the filter; 100 c.c. of the clear filtrate are diluted with 100 c.c. of water, 50 c.c. of nitric acid (*d*) and 10 c.c. of ferric-alum solution (*e*) are added, and the solution titrated with $n/10$ silver nitrate (*a*) until the red color is discharged. The addition of silver solution is continued

* Pflüger's Archiv, **69**, 423.

to the next even number of c.c., and then the solution titrated back with $n/10$ thiocyanate until the white liquid just begins to turn pink.

Let $A$ be the cubic centimeters of $n/10$ thiocyanate added to the 300 c.c. of solution, $B$ the cubic centimeters of $n/10$ silver nitrate added to the 100 c.c. of filtrate, and $C$ the cubic centimeters of $n/10$ thiocyanate to titrate back excess of $B$.

Since 1 c.c. $n/10$ thiocyanate = 6.357 mgs. copper then the total milligrams of copper (Cu) are found by the formula $\text{Cu} = 6.357\,(A + 3C - 3B)$. The thiocyanate solution should be standardized against pure copper under the conditions of analysis, as in the permanganate and iodide methods.

**Volumetric Cyanide Method.**—Of other volumetric processes which are used for determining reduced copper may be mentioned the well-known cyanide method. The unreduced copper in the filtrate from the cuprous oxide is titrated with standard potassium cyanide solution until the blue color disappears. The difference between the copper in the volume of Fehling's solution taken, and that found in the filtrate after reduction, is the amount of copper reduced by the sugar.

**Determination of Copper by Weighing as Cupric Oxide.** — In this method the cuprous oxide, after collecting upon a Gooch crucible, is heated to redness for about 15 minutes, when it is converted to black cupric oxide. To insure complete oxidation care must be taken that the oxide is not exposed to the reducing action of the illuminating gas during ignition. For this reason the operation is best carried out in a muffle.

If porcelain Gooch crucibles are used they should have open bottoms with loose perforated disks for supporting the asbestos (Caldwell's crucible, Fig. 174). The one-piece porcelain Gooch crucible is liable to crack at high temperatures of ignition.

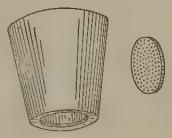


Fig. 174.—Gooch crucible with detachable bottom.

Finely-divided cupric oxide is hygroscopic and, after cooling in a desiccator, should be weighed as quickly as possible. The weight of cupric oxide multiplied by the factor 0.7989 gives the weight of metallic copper. Several sugar tables, as Kjeldahl's and Defren's, express results in terms of cupric oxide, thus avoiding the labor of calculation, when this method of determining copper is used.

The method of estimating copper from the weight of cupric oxide is one of the most accurate of the indirect methods. With impure products, however, the precipitate of cuprous oxide frequently carries

down mineral matter and this contamination will impair somewhat the accuracy of the method (see Table LXXIII).

**Determination of Copper by Direct Weighing of the Cuprous Oxide.** — In this method the precipitated cuprous oxide is collected in a filter tube or Gooch crucible in the usual way. Wash thoroughly with hot water, then with 10 c.c. of alcohol and finally with 10 c.c. of ether. Dry the precipitate 30 minutes in a water oven at the temperature of boiling water; cool and weigh. The weight of cuprous oxide multiplied by 0.8882 gives the weight of metallic copper. The sugar tables of Munson and Walker express results in terms of cuprous oxide, and the use of these tables will save much labor of calculation when this method of determining copper is used.

*Contamination of Cuprous Oxide.* — Direct weighing of the cuprous oxide is the simplest of the gravimetric methods for estimating reduced copper in sugar analysis. The process, however, is less accurate than the other methods previously described. The method gives good results with sugar solutions of high purity, but with impure products the cuprous oxide is contaminated with mineral and organic impurities, which may affect considerably the accuracy of the determination.

The extent of the error in estimating copper from the weight of cuprous oxide is shown by the following comparative analyses made by Sherwood and Wiley * upon a variety of sugar-containing products.

TABLE LXXIII

*Comparison of Methods for Determining Reduced Copper*

| Material. | Reduced Copper. | | |
|---|---|---|---|
| | From weight of cuprous oxide | From weight of cupric oxide. | Volumetric iodide method (Low). |
| | Gram. | Gram. | Gram. |
| Molasses residuum | 0.3753 | 0.3594 | 0.3494 |
| " " | 0.3905 | 0.3634 | 0.3470 |
| " " | 0.2517 | 0.2348 | 0.2242 |
| " " | 0.3287 | 0.3130 | 0.3034 |
| " " | 0.3291 | 0.3134 | 0.3029 |
| " " | 0.2768 | 0.2698 | 0.2688 |
| " " | 0.2709 | 0.2620 | 0.2612 |
| Pure dextrose | 0.4619 | ..... | 0.4617 |
| " " | 0.2449 | ..... | 0.2444 |
| " " | 0.1251 | ..... | 0.1257 |
| Beer | 0.0755 | ..... | 0.0753 |
| " | 0.0746 | ..... | 0.0748 |
| Molasses | 0.4628 | ..... | 0.4520 |
| Corn juice | 0.3360 | ..... | 0.3134 |
| Malt extract | 0.3322 | ..... | 0.3048 |
| " " | 0.3160 | ..... | 0.2933 |
| " " | 0.2093 | ..... | 0.1934 |

* Bull. 105, U. S. Bur. of Chem., p. 120.

The results upon the molasses residuum show a contamination of the cuprous oxide with organic matter as shown by the differences in copper as calculated from the suboxide and oxide, and with mineral matter as shown by the differences in copper as calculated from the oxide and by the volumetric method.

With solutions of pure sugar and such liquids as beer, where the organic matter consisted largely of carbohydrates, the calculation of copper from the weight of cuprous oxide gave accurate results. In the case of the malt extracts, which contained added peptones, the precipitated cuprous oxide seemed to carry down a considerable amount of albuminoid matter from solution; in the case of the molasses the precipitated copper seemed to be in partial combination with certain nitrogenous bases such as xanthine.

Similar comparisons upon methods of determining copper in the analysis of cane-sugar products are given in Table LXXX.

The chemist is usually able to form an opinion of the purity of the cuprous oxide from its physical appearance. If the precipitate is yellow or greenish-red in color, or has a flaky appearance, there is evidence of contamination, in which case the reduced copper must be determined by one of the direct methods.

### CAUSES AFFECTING THE ACCURACY OF ESTIMATING SUGARS FROM A DETERMINATION OF REDUCED COPPER

In addition to the errors in determining reduced copper, there are a number of other causes which affect the accuracy of the analytical methods belonging to this class.

**Purity of Reagents.** — A frequent cause of inaccuracy in determining sugars by the methods of copper reduction is the presence of organic or mineral impurities in the Fehling's solution. The copper sulphate, the caustic alkali and especially the Rochelle salts should be of the purest quality. The copper sulphate and alkali-tartrate solutions should be filtered separately through glass wool, or asbestos, and the mixed reagent should be perfectly clear and show no trace of cuprous oxide after boiling. A blank determination should be made upon each fresh lot of solution; the crucibles, or filter tubes, used in the blank test should show no increase in weight under the conditions of experiment followed in analysis.

**Degree of Dilution and Time of Boiling.** — The effect of varying the dilution of Fehling's solution, or the time of boiling, is shown by the following comparison of results from Allihn's table with those

obtained by Wein's, and by Koch and Ruhsam's modifications of Allihn's method.

| Reduced copper. | 2 minutes' heating. | | 30 minutes' heating. |
|---|---|---|---|
| | Diluted (Allihn). Glucose. | Undiluted (Wein). Glucose. | Diluted (Koch and Ruhsam). Glucose. |
| Mgs. | Mgs. | Mgs. | Mgs. |
| 10 | 6.1 | 4.5 | 4.1 |
| 50 | 25.9 | 24.6 | 21.3 |
| 100 | 50.9 | 49 9 | 46.9 |
| 150 | 76.5 | 75.5 | 72.0 |
| 200 | 102.6 | 101.7 | 96.8 |
| 250 | 129.2 | 128.3 | 122.7 |
| 300 | 156.5 | 155.6 | 149.0 |
| 350 | 184.3 | 182.3 | 176.2 |
| 400 | 212.9 | 212.0 | 205.0 |
| 450 | 242.2 | 240.6 | 235.9 |

It is seen that considerably more copper is reduced by using a more concentrated Fehling's solution or by heating for a longer time.

Incomplete reduction of the copper solution has been raised as an objection against such methods as Allihn's, which boil for only 2 minutes. If the time of filtration be too prolonged an additional amount of copper is sometimes precipitated, thus increasing the results. It is important, therefore, with methods which boil for only 2 minutes to filter immediately, and as rapidly as permissable, at the end of the time limit.

**Atmospheric Pressure and Temperature of Boiling.** — Variable temperature of boiling, due to difference in altitude above sea-level, has been suggested by Traphagen and Cobleigh * as a cause of differences in determining reducing sugars. Rosenkranz † has recently studied the influence of pressure upon the reducing power of invert sugar with the following results:

| Pressure. | Temperature of boiling. | 25 c.c. invert sugar solution plus | |
|---|---|---|---|
| | | 25 c.c. water. 50 c.c. Fehling's solution. | 25 c.c. 10% sucrose solution. 50 c.c. Fehling's solution. |
| Millimeters. | Deg. C. | Mgs. Cu. | Mgs. Cu. |
| { 775 | 103–105 | 236.5 | 260.4 |
| { 600 | 90– 96 | 232.5 | 244.9 |
| { 760 | 103–104 | 235.6 | 277.7 |
| { 925 | 109–110 | 236.1 | 296.3 |

* J. Am. Chem. Soc., **21**, 369. † Z. Ver. Deut. Zuckerind., **61** (1911), 426.

The results show for pure invert sugar a slight tendency towards increase in reducing power with increase in pressure; the error due to this cause, however, is slight and may be neglected for ordinary atmospheric conditions. When sucrose is present the increase in pressure causes a marked increase in the amount of reduced copper, owing to the much greater degree of inversion.

**Surface Area of Solution.** — The diameter of the vessel in which the Fehling's solution is heated has been found to influence the amount of reduced copper. With wide beakers, which expose a larger area of solution to the air, more cuprous oxide is lost by oxidation (through being redissolved in the alkaline-tartrate solution) than in narrower beakers. Kjeldahl has eliminated the error due to oxidation by making the reduction in an atmosphere of hydrogen or of oxygen-free illuminating gas.

Under the same set of conditions the oxidation error is a constant one and the discrepancies due to this cause are eliminated by making the reduction always in beakers of the same size. A 350–400-c.c. lipped beaker of Jena, or Non-sol glass, 7–8 cm. in diameter is about the proper size.

### SPECIAL COPPER–REDUCTION METHODS

**Modifications of Allihn's Method.** — Allihn's method gives the most accurate results upon sugar solutions containing 0.4 to 1.0 per cent glucose, i.e., 0.10 to 0.25 gm. glucose in the 25 c.c. of solution. When less than 50 mgs. of glucose are present the method is apt to show wide discrepancies in the hands of different chemists. Several modifications of Allihn's method, involving a longer period of heating, have been devised for the purpose of increasing the accuracy of the determination with dilute sugar solutions.

**Pflüger's Method.** — Pflüger,* who uses the same reagents and volumes of solutions as in Allihn's method, has modified the determination by heating the mixed sugar and Fehling's solutions (145 c.c. in all) in a boiling-water bath for exactly 30 minutes and then diluting with 130 c.c. of cold water before filtering. The cuprous oxide is filtered upon asbestos and, after washing and drying, the weight of precipitate determined. Owing to the frequent occurrence of impurities in the cuprous oxide, especially when working with fluids or extracts of animal origin, Pflüger advises to make also a direct determination of the copper by means of the thiocyanate method.

* Pflüger's Archiv, **69**, 399.

Pflüger's table giving the weights of glucose corresponding to different weights of cuprous oxide and copper, is found in the Appendix (Table 11).

**Koch and Ruhsam's * Method.** — In this modification the same reagents and volumes of solutions are used as in Allihn's and Pflüger's methods. The mixed sugar and Fehling's solutions (145 c.c. in all) are first brought to a boil and then set in a boiling-water bath for exactly 30 minutes. The solution without diluting is then filtered through asbestos in a Gooch crucible and the reduced copper determined by any of the usual methods.

The glucose table for Koch and Ruhsam's method is given in the Appendix (Table 12).

Koch and Ruhsam's modification was designed for determining glucose in tannin extracts, etc., and is the official method of the American Leather Chemists and other similar associations.

The modifications of Allihn's method, using 30-minute heating, are considerably more accurate than the original process upon dilute glucose solutions and should be employed for determining small amounts of sugar in urine, tannin extracts and other animal and vegetable substances of low glucose content. When, however, the 25 c.c. of sugar solution contain over 0.10 gm. of glucose, Allihn's original method of 2-minute boiling may be followed with perfect safety, and with a considerable economy of time. The fact that more copper is reduced upon longer heating does not affect the accuracy of the method, since the tables were standardized under exactly similar conditions.

**Application of Allihn's Method to the Determination of Other Reducing Sugars.** — Allihn's method has been employed for determining other reducing sugars besides glucose. Hönig and Jesser † have used the method for determining fructose and have constructed a table giving the copper-reducing power of fructose for different weights of sugar. In Table LXXIV the fructose values of Hönig and Jesser, and the corresponding glucose values of Allihn, are given for several weights of reduced copper. The ratio of fructose to glucose, for the same weight of copper, is also given.

For equal weights of sugar the amount of copper reduced by fructose is about 92 per cent of that reduced by glucose. Soxhlet found by his volumetric method (p. 391) that for equal weights of sugar the reducing power of fructose was 92.4 per cent that of glucose.

* J. Soc. Chem. Ind., **13**, 1227.
† Monatshefte, **9**, 562.

TABLE LXXIV

*Showing Comparative Reducing Power of Fructose and Glucose*

| Reduced copper. | Fructose (Hönig and Jesser). | Glucose (Allihn). | Ratio $\frac{\text{glucose}}{\text{fructose}}$. |
|---|---|---|---|
| Mgs. | | | |
| 32.7 | 20 | 17.4 | 0.870 |
| 70.2 | 40 | 35.9 | 0.898 |
| 107.1 | 60 | 54.6 | 0.910 |
| 143.2 | 80 | 73.0 | 0.912 |
| 178.9 | 100 | 91.5 | 0.915 |
| 213.9 | 120 | 110.0 | 0.917 |
| 248.3 | 140 | 128.3 | 0.916 |
| 282.2 | 160 | 146.7 | 0.917 |
| 315.3 | 180 | 165.0 | 0.917 |
| 347.9 | 200 | 183.1 | 0.916 |
| 379.9 | 220 | 201.3 | 0.915 |
| 411.3 | 240 | 219.5 | 0.915 |
| Average ratio (excluding first 2 of the series) | | | 0.915 |

**Reducing Ratios of Sugars.** — It is seen from Table LXXIV that if the values are eliminated for weights of sugar under 50 mgs., for which, as previously stated, Allihn's method gives uncertain results, the ratio of fructose to glucose for the same weight of reduced copper is a constant quantity 0.915. Other monosaccharides show a similar constancy of ratio. The following ratios are given by Browne * for a number of other sugars, the copper-reducing power in all cases being determined by Allihn's method:

$$\frac{\text{Glucose}}{\text{Arabinose}} = 1.032.$$

$$\frac{\text{Glucose}}{\text{Xylose}} = 0.983.$$

$$\frac{\text{Glucose}}{\text{Invert Sugar}} = 0.958.$$

$$\frac{\text{Glucose}}{\text{Galactose}} = 0.898.$$

**Relative Copper-reducing Power.** — Instead of using the ratios of the weights of sugars for the same amount of reduced copper, the ratios of the weights of copper reduced by the same amount of the two sugars are frequently used. O'Sullivan † expressed the relative copper-reducing power of a sugar by the symbol $K$ and adopted as his standard ($K = 100$) the cupric oxide reduced by a given weight of glucose under the conditions of his method. O'Sullivan found, for example, that 1 gm.

* J. Am. Chem. Soc., **28,** 439.

† J. Chem. Soc. (1879), 72, 275.

of glucose reduced 2.205 gms. CuO and 1 gm. of maltose 1.345 gms. CuO. The relative copper, or cupric oxide, reducing power of maltose would then be $K = \frac{1.345}{2.205} \times 100 = 61.$

In the examination of starch-conversion products the copper-reducing power of maltose, expressed by the symbol $R$, is sometimes taken as the standard. Taking the previous values of O'Sullivan the $R$ of glucose would be $\frac{2.205}{1.345} \times 100 = 164.$

In place of the constant $K$, Brown, Morris and Millar * have substituted the value $\kappa$, which is $\frac{1}{100} K$. According to this system the relative copper-reducing power of maltose (using O'Sullivan's results) is 0.61 $\kappa$. The values for $\kappa$, when determined for the same absolute weights of the two sugars, are practically identical with the reducing ratios as calculated in the previous section.

Thus from Defren's table for glucose and maltose 44.4 mgs. of glucose reduce 100 mgs. CuO and 44.4 mgs. of maltose reduce 61.1 mgs. CuO then $\frac{61.1}{100} = 0.611$, $\kappa$ for maltose.

Using again Defren's table 44.4 mgs. glucose and 72.8 mgs. maltose reduce respectively 100 mgs. CuO, then $\frac{44.4}{72.8} = 0.610$, the reducing ratio of maltose to glucose.

If $\kappa$, however, is calculated from the weights of sugars as determined by the solution factor 3.86, as is sometimes done, then the true reducing ratio is not found unless a correction be applied as indicated on page 32.

The disaccharides, lactose and maltose, do not show usually the same constancy in reducing ratios for different weights of copper as the monosaccharides. This is due to the partial hydrolysis of the disaccharides as previously explained; the reducing ratio is usually higher the greater the amount of disaccharide. The copper-reducing ratios of lactose and maltose are approximately as follows for Allihn's method:

$$\frac{\text{Glucose}}{\text{Lactose hydrate}} = 0.66 \text{ to } 0.71, \text{ or approximately } 0.7.$$

$$\frac{\text{Glucose}}{\text{Maltose}} = 0.56 \text{ to } 0.62, \text{ or approximately } 0.6.$$

* J. Chem. Soc. (1897), 96.

If the copper-reducing power of a sugar is determined (as by Allihn's method), the corresponding glucose value of Allihn's table divided by the reducing ratio of the sugar to glucose will give the weight of sugar in the 25 c.c. of solution.

*Example.* — 25 c.c. of a fructose solution gave by Allihn's method 265.3 mgs. of copper.

The amount of glucose corresponding to 265.3 mgs. of copper, according to Allihn's table, is 137.45 mgs. 137.45 ÷ 0.915 (the reducing ratio of fructose to glucose) = 150.2 mgs. of fructose. The amount of fructose corresponding to 265.3 mgs. of copper according to Hönig and Jesser is 150 mgs.

The reducing ratios of the different sugars, have an important bearing upon the analysis of sugar mixtures, as described in Chapter XVI.

Special copper-reduction methods and tables, similar to those of Allihn, have been established for other reducing sugars. It is impossible to describe all of these in detail and only the following examples are given for invert sugar, maltose and lactose. The methods and tables are taken from Wein's "Zuckertabellen."

**Meissl's* Method for Determining Invert Sugar.** — The Soxhlet formula for Fehling's solution is used; 25 c.c. of the copper-sulphate solution and 25 c.c. of the alkaline-tartrate solution are mixed with the sugar solution, which should not contain over 0.245 gm. of invert sugar. Enough water is added to make the whole up to 100 c.c., the liquid is heated to boiling and kept at ebullition for exactly 2 minutes. The cuprous oxide is then filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of invert sugar corresponding to different weights of reduced copper are given in the Appendix in Table 13, which was calculated by Wein from Meissl's reduction factors.

**Wein's† Method for Determining Maltose.** — The Soxhlet formula for Fehling's solution is used; 25 c.c. of the copper-sulphate solution and 25 c.c. of the alkaline-tartrate solution are mixed and heated to boiling; 25 c.c. of the sugar solution, which should not contain over 0.25 gm. of maltose, are then added and the liquid boiled for exactly 4 minutes. The cuprous oxide is filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of maltose corresponding to different weights of reduced copper are given in the Appendix in Table 14.

According to Brown, Morris and Millar,‡ whose results have been

* Z. Ver. Deut. Zuckerind., **29**, 1050.

† Wein's "Tabellen." ‡ J. Chem. Soc., Trans., **71**, 96.

confirmed by Ling and Baker,* the table of Wein gives results which are about 5 per cent too low.

**Soxhlet's† Method for Determining Lactose.** — The Soxhlet formula for Fehling's solution is used; 25 c.c. of the copper-sulphate solution and 25 c.c. of the alkaline-tartrate solution are mixed with 20 to 100 c.c. (according to concentration) of the milk-sugar solution, which should not contain over 0.300 gms. of lactose hydrate. If less than 100 c.c. of milk-sugar solution is taken sufficient water is added to make the whole up to 150 c.c. The liquid is then heated to boiling and kept at ebullition for exactly 6 minutes. The cuprous oxide is filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of lactose hydrate corresponding to different weights of reduced copper are given in the Appendix in Table 15, calculated by Wein from Soxhlet's reduction factors.

### UNIFIED COPPER-REDUCTION METHODS FOR SEVERAL SUGARS

The confusing multiplicity of copper-reducing tables is due to the fact that different investigators have confined their work to one single sugar for one individual set of conditions. A number of chemists, however, have worked with the purpose of establishing one uniform method for all reducing sugars. Examples of such unified methods are those of Kjeldahl and Woy, Defren, Munson and Walker, and Bertrand.

**Unified Method of Kjeldahl‡ and Woy.§** — In Kjeldahl's method, as modified by Woy, the Fehling's solution is prepared for each analysis with a freshly weighed portion of Rochelle salts. The following solutions are used:

(*A*) 69.278 gms. of pure $CuSO_4.5\ H_2O$ are dissolved to 1000 c.c.

(*B*) 130 gms. of pure sodium hydroxide (the amount must be established by titration) are dissolved to 1000 c.c.

According to the richness of the sugar solution, 15 c.c., 30 c.c. or 50 c.c. of mixed reagent are made up in an Erlenmeyer flask holding about 150 c.c.

For 15 c.c. of reagent take 7.5 c.c. of *A*, 7.5 c.c. of *B* and 2.6 gms. Rochelle salts.

For 30 c.c. of reagent take 15.0 c.c. of *A*, 15.0 c.c. of *B* and 5.2 gms. Rochelle salts.

For 50 c.c. of reagent take 25.0 c.c. of *A*, 25.0 c.c. of *B* and 8.65 gms. Rochelle salts.

The sugar solution is then added, the total volume of liquid in the

* J. Chem. Soc., Trans., **71,** 509.
† J. prakt. Chem., **21,** 266.
‡ Neue Z. Rübenzuckerind., **37,** 29.
§ Chem. Centralblatt. **97** [2], 986.

flask being always brought to 100 c.c. The flask is then plunged in a boiling-water bath and heated for exactly 20 minutes, while leading through the liquid a stream of hydrogen, or of illuminating gas which has been freed of oxygen by passing through a gas washer containing pyrogallic acid and sodium hydroxide solution. The reoxidation of the cuprous oxide by the air is in this way prevented. At the end of the 20 minutes the cuprous oxide is filtered on asbestos, washed, ignited and weighed as cupric oxide. The amounts of glucose, fructose, invert sugar, lactose hydrate or maltose corresponding to different weights of cupric oxide or copper are given in the Appendix in Table 16, which was calculated by Woy for the 15-c.c., 30-c.c. and 50-c.c. volumes of reagent.

The Kjeldahl-Woy method is one of great exactness, being carried out under rigidly defined conditions. The rather complicated details in preparing the copper reagent and in conducting the reduction have prevented the process from coming into extensive use.

**Unified Method of Brown, Morris and Millar.*** — In this method, which is adapted from a previous process by O'Sullivan, the Fehling's solution is prepared by dissolving 34.6 gms. crystallized copper sulphate, 173 gms. Rochelle salts and 65 gms. anhydrous sodium hydroxide to 1000 c.c.; 50 c.c. of the reagent are placed in a beaker of about 250 c.c. capacity and of 7.5 cm. diameter. The beaker is set in a boiling-water bath, and when the solution has acquired the same temperature, the measured volume of sugar solution is added and the whole made up to 100 c.c. with boiling distilled water. The beaker is covered with a clock glass, returned to the bath and heated exactly 12 minutes. The cuprous oxide is filtered in a tube and weighed as metallic copper or cupric oxide.

The table of Brown, Morris and Millar (Appendix, Table 17) gives the weight of copper and cupric oxide which correspond to the same weight of glucose, fructose and invert sugar, the order of arrangement being the reverse of that in most tables.

**Unified Method of Defren.†** — In Defren's method, which is adapted from O'Sullivan, Soxhlet's formula for Fehling's solution is used; 15 c.c. of the copper-sulphate solution and 15 c.c. of the alkaline-tartrate solution are diluted with 50 c.c. of water in a 300-c.c. Erlenmeyer flask. The latter is then immersed for 5 minutes in a boiling-water bath, when 25 c.c. of the sugar solution are quickly run in from a burette. The flask is replaced in the bath and heated for exactly 15 minutes. The cuprous oxide is then filtered on asbestos, washed,

* J. Chem. Soc., Trans., **71**, 281.

† J. Am. Chem. Soc., **18**, 751.

ignited and weighed as cupric oxide. The amounts of glucose, maltose or lactose corresponding to different weights of cupric oxide are given in the Appendix in Table 18.

**Unified Method of Munson and Walker.*** — Transfer 25 c.c. each of the copper and alkaline-tartrate solutions (Soxhlet's formula) to a 400-c.c. Jena or Non-sol beaker and add 50 c.c. of reducing sugar solution, or, if a smaller volume of sugar solution be used add water to make the final volume 100 c.c. Heat the beaker upon an asbestos gauze over a Bunsen burner; so regulate the flame that boiling begins in 4 minutes, and continue the boiling for exactly 2 minutes. Keep the beaker covered with a watch glass throughout the entire time of heating. Without diluting filter the cuprous oxide at once on an asbestos felt in a porcelain Gooch crucible, using suction. Wash the cuprous oxide thoroughly with water at a temperature of about 60° C., then with 10 c.c. of alcohol and finally with 10 c.c. of ether. Dry for 30 minutes in a water oven at 100° C., cool in a desiccator and weigh as cuprous oxide. The amounts of glucose, invert sugar, lactose or maltose corresponding to different weights of cuprous oxide or copper are given in the Appendix in Table 19.

**Unified Method of Bertrand.†** — The following formula is used in preparing the copper reagents:

(*A*) 40 gms. of pure $CuSO_4.5\ HO_2$ are dissolved to 1000 c.c.,

(*B*) 200 gms. of Rochelle salts and 150 gms. of sodium hydroxide in sticks are dissolved to 1000 c.c.:

20 c.c. of the sugar solution, which should not contain over 0.100 gm. of reducing sugars, are transferred to a 150-c.c. Erlenmeyer flask, and 20 c.c. each of solutions *A* and *B* added. The liquid is then heated to boiling and kept at gentle ebullition for exactly 3 minutes. The solution is then filtered through asbestos, the precipitate of cuprous oxide washed with distilled water and the reduced copper determined by the volumetric permanganate method.

The table of Bertrand (Appendix, Table 20) gives the different weights of reduced copper which correspond to the same weight of invert sugar, glucose, galactose, maltose and lactose, the order of arrangement being the same as in the table of Brown, Morris and Millar.

### METHODS FOR DETERMINING REDUCING SUGARS IN PRESENCE OF SUCROSE

Reference has been made to the slight hydrolytic action of hot Fehling's solution upon the higher saccharides. While this action in

* J. Am. Chem. Soc., **28,** 663; **29,** 541; **34,** 202. † Bull. soc. chim., **35,** 1285.

case of sucrose is slight it is, nevertheless, sufficiently pronounced to cause a considerable error in the determination of reducing sugars when much sucrose is present.

**Conditions Affecting the Reducing Action of Sucrose upon Fehling's Solution.** — The reducing action of sucrose upon Fehling's solution is proportional first, to the concentration of the sucrose and, second, to the amount of copper left unreduced. If enough reducing sugars are present to precipitate nearly all the copper from the Fehling's solution the inversion of the sucrose will be very slight. This is shown in Table LXXV, which gives a series of experiments by Browne.* Constant quantities of sucrose, and varying amounts of glucose, were taken, and a determination of the latter made by Allihn's method.

TABLE LXXV

*Showing Influence of Sucrose Upon the Reducing Action of Glucose*

| *A.* Sucrose taken in 25 c.c. | *B.* Glucose taken in 25 c.c. | *C.* Glucose found in 25 c.c. | *D.* Error $(C - B)$. | *E.* Calculated correction, $\left(\frac{\text{mgs. sucrose}}{\text{mgs. glucose} + 40}\right)$ | *F.* Corrected glucose, $(C - E)$. |
|---|---|---|---|---|---|
| Mgs. | Mgs. | Mgs. | Mgs. | Mgs. | Mgs. |
| 250 | 50 | 52.3 | 2.3 | 2.7 | 49.6 |
| 250 | 100 | 102.8 | 2.8 | 1.8 | 101.0 |
| 250 | 150 | 151.8 | 1.8 | 1.3 | 150.5 |
| 250 | 200 | 199.0 | −1.0 | 1.0 | 198.0 |
| 500 | 100 | 104.5 | 4.5 | 3.5 | 101.0 |
| 500 | 150 | 153.2 | 3.2 | 2.6 | 150.6 |
| 500 | 200 | 203.2 | 3.2 | 2.1 | 201.1 |
| 500 | 250 | 251.3 | 1.3 | 1.7 | 249.6 |
| 1000 | 50 | 60.3 | 10.3 | 10.0 | 50.3 |
| 1000 | 100 | 108.2 | 8.2 | 6.8 | 101.4 |
| 1000 | 200 | 205.3 | 5.3 | 4.1 | 201.2 |
| 1000 | 250 | 252.0 | 2.0 | 3.4 | 248.6 |
| 2000 | 50 | 66.6 | 16.6 | 18.8 | 47.8 |
| 2000 | 100 | 113.7 | 13.7 | 13.0 | 100.7 |
| 2000 | 200 | 207.5 | 7.5 | 8.1 | 199.4 |
| 2000 | 250 | 255.5 | 5.5 | 6.8 | 248.7 |

The error in the glucose determination, when sucrose is present, is seen to be considerable; it is even more pronounced in such reduction methods as those of Kjeldahl or Pflüger, which employ a long period of heating.

It is seen from Table LXXV that the error in the glucose determination is directly proportional to the amount of sucrose, and inversely proportional to the amount of glucose. Browne has proposed the use of an empirical formula, $\frac{\text{milligrams sucrose}}{\text{milligrams glucose} + 40}$, as a means of correct-

* J. Am. Chem. Soc., **28**, 451.

ing for the reducing action of sucrose, when using Allihn's method. Table LXXV gives a comparison of the actual errors and of the results corrected by means of such a formula.

In the volumetric methods of Soxhlet, Violette, etc., where the invert sugar solution is added to the point of complete reduction, no excess of copper is left in solution, and the error due to the presence of sucrose is practically negligible.

A number of special copper-reduction methods have been designed for determining invert sugar in sugar-house products. The methods are classified according to the excess of sucrose over invert sugar in the material to be analyzed.

**Herzfeld's* Method for Determining Invert Sugar in Raw Sugars Containing Less than 1.5 per cent Invert Sugar.** — This method is designed for the analysis of the higher grades of raw sugar. The sugar solution, which should contain 20 gms. of material in 100 c.c. and be free from suspended or soluble impurities, is conveniently prepared as follows:

Dissolve 44 gms. of sugar in about 100 c.c. of water in a 200-c.c. graduated flask. A little normal lead-acetate solution, just sufficient for clarification, is then added and the volume completed to 200 c.c. The solution is shaken, filtered and 100 c.c. of the filtrate (22 gms. sugar) measured into a 100–110 c.c. flask. Sufficient carbonate, or sulphate of sodium is then added to precipitate the excess of lead and the volume made up to 110 c.c. The solution is shaken, filtered and 50 c.c. of the filtrate (10 gms. of sugar) used for the determination.

Heat 25 c.c. each of the copper-sulphate and alkaline-tartrate solutions (Soxhlet's formula) to boiling; the 50 c.c. of clarified sugar solution are then added and the whole boiled for exactly 2 minutes. The cuprous oxide is filtered on asbestos, washed and the reduced copper determined by any of the usual methods. The amounts of invert sugar corresponding to different weights of copper are given in the Appendix, in Table 21.

In case the percentage of invert sugar in the raw sugar exceeds 1.5 per cent, Herzfeld's method is no longer applicable.

**Meissl and Wein's† Method for Determining Invert Sugar in Mixtures of 90 to 99 per cent Sucrose with 10 to 1 per cent Invert Sugar.** — This method is designed for the analysis of low-grade raw sugars, or of other sugar-house products which do not contain a large

* Z. Ver. Deut. Zuckerind. (1885), 985.

† Wein's "Tabellen."

excess of invert sugar. The sugar solution is prepared as in the previous method, the final filtrate being diluted if necessary so as not to contain more than 0.2 to 0.245 gms. of invert sugar in 50 c.c.

Mix 25 c.c. each of the copper-sulphate and alkaline-tartrate solutions (Soxhlet's formula) with the 50 c.c. of clarified sugar solution; the liquid is then heated to boiling and kept at gentle ebullition for exactly 2 minutes. The cuprous oxide is then filtered on asbestos, washed and the reduced copper determined by any of the usual methods.

For determining the weights of invert sugar corresponding to different weights of reduced copper, for percentages of sucrose between 90 and 99, the following condensed table has been calculated by Wein. Intermediary values can be easily calculated by interpolating.

TABLE LXXVI

*For Determining Invert Sugar in Presence of Sucrose.* (*Meissl and Wein.*)

| In mixtures of sucrose ($S$) and invert sugar ($I$) in parts per hundred. | Milligrams of invert sugar. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 245 | 225 | 200 | 175 | 150 | 125 | 100 | 75 | 50 |
| | Correspond to Milligrams of Copper. | | | | | | | | |
| 99 $S$ + 1 $I$ | ..... | ..... | 417.3 | 370.8 | 323.6 | 277.5 | 230.0 | 182.0 | 131.5 |
| 98 $S$ + 2 $I$ | ..... | ..... | 393.7 | 357.7 | 304.7 | 259.7 | 213.7 | 166.0 | 113.8 |
| 97 $S$ + 3 $I$ | ..... | ..... | 385.7 | 350.6 | 298.4 | 253.8 | 207.9 | 158.3 | 107.9 |
| 96 $S$ + 4 $I$ | ..... | ..... | 381.7 | 339.1 | 295.3 | 250.8 | 205.0 | 155.4 | 105.7 |
| 95 $S$ + 5 $I$ | 439.7 | 420.1 | 379.3 | 337.0 | 293.4 | 249.0 | 203.3 | 153.6 | 103.2 |
| 94 $S$ + 6 $I$ | 438.5 | 416.5 | 376.6 | 334.7 | 290.1 | 245.4 | 199.8 | 151.0 | 101.5 |
| 93 $S$ + 7 $I$ | 437.6 | 413.9 | 374.6 | 332.3 | 287.8 | 242.9 | 197.3 | 149.2 | 100.2 |
| 92 $S$ + 8 $I$ | 437.0 | 411.9 | 373.1 | 330.4 | 286.3 | 241.0 | 195.4 | 147.9 | 99.3 |
| 91 $S$ + 9 $I$ | 436.5 | 410.3 | 372.0 | 328.8 | 285.1 | 239.4 | 193.9 | 146.8 | 98.6 |
| 90 $S$ +10 $I$ | 436.1 | 409.2 | 371.1 | 327.8 | 284.0 | 238.2 | 192.7 | 146.0 | 98.0 |

The employment of the above table is best understood from an example:

A sugar, which indicated 96.2 per cent sucrose by Clerget's method, was made up so that 50 c.c. of the clarified and deleaded solution contained 10 gms. of sample. The amount of reduced copper obtained by Meissl's method was 324 mgs. Required the percentage of invert sugar.

The invert sugar corresponding to 324 mgs. copper according to Meissl's table for invert sugar alone is 178 mgs. or 1.78 per cent (uncorrected). The percentage composition, in a mixture of 96.2 parts sucrose with 1.78 parts invert sugar is approximately 98 per cent sucrose and 2 per cent invert sugar. Opposite the mixture 98 $S$ + 2 $I$ of the table it is seen that

357.7 mgs. of copper = 175 mgs. invert sugar,

and 304.7 mgs. of copper = 150 mgs. invert sugar,

then for the intermediary 324.0 mgs. of copper

$\frac{324.0 - 304.7}{357.7 - 304.7} = 0.36$. $(175 - 150) \times 0.36 = 9.0$ mgs. $150 + 9.0 = 159.0$ mgs. of invert sugar or 1.59 per cent.

Meissl and Wein's method is not applicable to products which contain more than 10 parts invert sugar in 100 parts of mixed sugars. For this reason the method has largely given place to the more general process of Meissl and Hiller.

**Meissl and Hiller's * Method for Determining Invert Sugar in Mixtures Containing less than 99 per cent Sucrose and more than 1 per cent Invert Sugar.** — This method is designed for the analysis of all sugar-house products except the highest grades of raw sugars. The method is based upon the principle of taking such a quantity of material for analysis that the invert sugar will reduce nearly all the copper, thus reducing the error due to presence of sucrose to a minimum.

The sugar solution is prepared as in the two previous methods so that 100 c.c., after clarification and deleading, contain 20 gms. of sample. Prepare a series of solutions in large test tubes by adding 1, 2, 3, 4 and 5 c.c. of this solution to each tube successively. Add 5 c.c. of the mixed copper reagent (Soxhlet's formula) to each, heat to boiling 2 minutes, and filter. Note the volume of sugar solution which gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar solution in a 100-c.c. flask, dilute to the mark and mix well. Use 50 c.c. of the solution for the determination, which is conducted as in the method of Meissl and Wein. The invert sugar is then calculated by means of the following formulæ.

Let Cu = the weight of copper obtained;

$P$ = the polarization of the sample;

$W$ = the weight of sample in the 50 c.c. of solution used for determination;

$F$ = the factor obtained from the table for conversion of copper to invert sugar;

$\frac{\text{Cu}}{2}$ = approximate weight of invert sugar = $A$;

$A \times \frac{100}{W}$ = approximate per cent of invert sugar = $y$;

$\frac{100\,P}{P + y}$ = $S$, approximate per cent of sucrose in mixture of sugars;

$100 - S = I$, approximate per cent of invert sugar;

$\frac{\text{Cu}\,F}{W}$ = per cent of invert sugar.

* Z. Ver. Deut. Zuckerind. (1889), 735.

The factor $F$ for calculating copper to invert sugar is then found from the following table:

TABLE LXXVII

*Meissl and Hiller's Factors for Calculating Copper to Invert Sugar for Different Ratios of Sucrose to Invert Sugar*

| Ratio of sucrose to invert sugar $= S : I$. | Approximate weight of invert sugar $= A$. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 200 Mgs. | 175 Mgs. | 150 Mgs. | 125 Mgs. | 100 Mgs. | 75 Mgs. | 50 Mgs. |
| 0 : 100 | 56.4 | 55.4 | 54.5 | 53.8 | 53.2 | 53.0 | 53.0 |
| 10 : 90 | 56.3 | 55.3 | 54.4 | 53.8 | 53.2 | 52.9 | 52.9 |
| 20 : 80 | 56.2 | 55.2 | 54.3 | 53.7 | 53.2 | 52.7 | 52.7 |
| 30 : 70 | 56.1 | 55.1 | 54.2 | 53.7 | 53.2 | 52.6 | 52.6 |
| 40 : 60 | 55.9 | 55.0 | 54.1 | 53.6 | 53.1 | 52.5 | 52.4 |
| 50 : 50 | 55.7 | 54.9 | 54.0 | 53.5 | 53.1 | 52.3 | 52.2 |
| 60 : 40 | 55.6 | 54.7 | 53.8 | 53.2 | 52.8 | 52.1 | 51.9 |
| 70 : 30 | 55.5 | 54.5 | 53.5 | 52.9 | 52.5 | 51.9 | 51.6 |
| 80 : 20 | 55.4 | 54.3 | 53.3 | 52.7 | 52.2 | 51.7 | 51.3 |
| 90 : 10 | 54.6 | 53.6 | 53.1 | 52.6 | 52.1 | 51.6 | 51.2 |
| 91 : 9 | 54.1 | 53.6 | 52.6 | 52.1 | 51.6 | 51.2 | 50.7 |
| 92 : 8 | 53.6 | 53.1 | 52.1 | 51.6 | 51.2 | 50.7 | 50.3 |
| 93 : 7 | 53.6 | 53.1 | 52.1 | 51.2 | 50.7 | 50.3 | 49.8 |
| 94 : 6 | 53.1 | 52.6 | 51.6 | 50.7 | 50.3 | 49.8 | 48.9 |
| 95 : 5 | 52.6 | 52.1 | 51.2 | 50.3 | 49.4 | 48.9 | 48.5 |
| 96 : 4 | 52.1 | 51.2 | 50.7 | 49.8 | 48.9 | 47.7 | 46.9 |
| 97 : 3 | 50.7 | 50.3 | 49.8 | 48.9 | 47.7 | 46.2 | 45.1 |
| 98 : 2 | 49.9 | 48.9 | 48.5 | 47.3 | 45.8 | 43.3 | 40.0 |
| 99 : 1 | 47.7 | 47.3 | 46.5 | 45.1 | 43.3 | 41.2 | 38.1 |

The use of Meissl and Hiller's formulæ and table for calculating invert sugar is best understood from an example.

The polarization of a sugar was 86.4; 50 c.c. of a solution containing 3.256 gms. of sample, reduced by Meissl and Hiller's method, 0.290 gms. of copper. Required the per cent of invert sugar.

$$\frac{\text{Cu}}{2} = \frac{0.290}{2} = 0.145 = A.$$

$$A \times \frac{100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = y.$$

$$\frac{100\,P}{P + y} = \frac{8640}{86.4 + 4.45} = 95.1 = S.$$

$$100 - S = 100 - 95.1 = I = 4.9.$$

$$S : I = 95.1 : 4.9.$$

By consulting the table it is seen that the vertical column headed 150 is nearest to $A$, 145, and the horizontal column having the ratio 95 : 5 is nearest to the ratio of $S$ to $I$, 95.1 : 4.9. At the intersection of these columns is found the factor 51.2 which enters into the final calculation $\frac{\text{Cu}\,F}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56$ per cent of invert sugar.

**Munson and Walker's * Method for Determining Invert Sugar in Presence of Sucrose.** — Munson and Walker have included in their unified method for reducing sugars determinations of invert sugar in presence of variable amounts of sucrose. Their table (Appendix, Table 19) gives the weight of invert sugar for different weights of cuprous oxide or copper, when the total weight of invert sugar and sucrose in the solution taken is 0.4 gm. and 2.0 gms. The 0.4 gm. amount is used preferably for sugar products containing between 1 and 9 parts of sucrose to 1 part of invert sugar and the 2.0 gms. amount for sugar products containing over 9 parts of sucrose to 1 part of invert sugar. This range is sufficient to include all the products of the sugar factory.

The method requires a preliminary investigation of the material in order to determine the approximate percentages of sucrose and invert sugar for use in making up the solution.

### MISCELLANEOUS COPPER-REDUCTION METHODS

The large amount of free alkali in Fehling's copper solution has proved its most objectionable feature, owing to the influence which it has in rendering sucrose and other substances slightly copper reducing. Attempts have accordingly been made to devise a copper reagent for sugar analysis which would contain no caustic alkali. While none of the solutions thus designed has shown the same all around suitability as that of Fehling, a few of them have found a certain usefulness in special cases.

**Barfoed's † Copper-acetate Method.** — Barfoed's copper-acetate solution (p. 336), which is not reduced by the disaccharides, maltose and lactose, has appealed to chemists as a convenient means of determining glucose, fructose and other monosaccharides in presence of the higher reducing sugars. But notwithstanding its value for qualitative purposes, attempts to use Barfoed's reagent for the quantitative determination of glucose and other monosaccharides have always given unsatisfactory results.

**Soldaini's ‡ Copper-bicarbonate Method.** — Soldaini's copper-bicarbonate solution (p. 337) has also appealed to chemists as a means of avoiding certain errors resulting from the use of Fehling's solution. Soldaini's method, however, has usually given unreliable results, when used for quantitative purposes, the principal objections being the deposition of copper hydroxide and the precipitation of lime and other mineral impurities with the reduced copper.

* J. Am. Chem. Soc., **28,** 663.
† Z. analyt. Chem., **12,** 27. ‡ Ber., **9,** 1126.

**Ost's * Copper-bicarbonate Method.** — Ost has modified Soldaini's reagent in order to eliminate its objectionable features. In his latest improvement of the method the copper reagent is prepared as follows: 250 gms. of chemically-pure potassium carbonate and 100 gms. of chemically-pure potassium bicarbonate are dissolved in water, and a solution containing 17.5 gms. of chemically-pure crystallized copper sulphate slowly added. The volume is then made up to 1000 c.c. and the solution filtered through asbestos, the first runnings of the filtrate being rejected.

In making the determination 100 c.c. of the copper reagent are treated with 50 c.c. of the sugar solution and the liquid boiled for 10 minutes. The precipitate is then filtered upon asbestos and the reduced copper determined by any of the usual methods.

Ost has unified his method for a number of reducing sugars; a few of the values for different weights of reduced copper are given in Table LXXVIII.

TABLE LXXVIII

*Showing Reducing Power of Different Sugars upon Ost's Copper Solution*

| Reduced copper. | Glucose. | Fructose. | Invert sugar. | Maltose. |
|---|---|---|---|---|
| Mgs. | Mgs. | Mgs. | Mgs. | Mgs. |
| 100 | 30.7 | 29.0 | 30.0 | 57.9 |
| 150 | 45.4 | 42.7 | 44.4 | 85.4 |
| 200 | 60.7 | 57.0 | 59 0 | 112.9 |
| 250 | 76.5 | 71.6 | 74.3 | 141.1 |
| 300 | 93.0 | 87.5 | 90.9 | 170.3 |
| 350 | 112.8 | 106.4 | 109.8 | 201.5 |
| 400 | 134.9 | 128.2 | 131.0 | 235.6 |

The method has not been found to give good results with lactose. Glucose, by Ost's process, reduces about 60 per cent more copper than by Allihn's method.

For determining small amounts of reducing sugars Ost recommends the use of his $\frac{1}{5}$-normal copper solution which contains 250 gms. chemically-pure potassium carbonate, 100 gms. chemically-pure potassium bicarbonate and 3.6 gms. chemically-pure crystallized copper sulphate to the liter. In using this solution, which is very sensitive towards small amounts of reducing sugars, the time of boiling is reduced to 5 minutes.

Ost's method has given good results in the analysis of pure sugar solutions, but has proved less reliable in the examination of low-grade products owing to the precipitation of lime and other mineral impurities.

* Chem. Ztg., **19**, 1784, 1829.

This difficulty, according to Ost, may be obviated by precipitating the lime with ammonium oxalate during the clarification. The method upon the whole has not offered sufficient advantages over Fehling's solution to come into general use.

**Bang's Copper-bicarbonate Method.** — Bang* has recently employed the copper-bicarbonate method for the volumetric determination of very small amounts of glucose. In this method the excess of copper, which remains in solution after reduction, is titrated with a standard hydroxylamine-sulphate solution in presence of potassium thiocyanate.

TABLE LXXIX

| Hydroxyl-amine. | Glucose. | Hydroxyl-amine. | Glucose. | Hydroxyl-amine. | Glucose. | Hydroxyl-amine. | Glucose. |
|---|---|---|---|---|---|---|---|
| c.c. | Mgs. | c.c. | Mgs. | c.c. | Mgs. | c.c. | Mgs. |
| 43.85 | 5 | 29.60 | 19 | 17.75 | 33 | 7.65 | 47 |
| 42.75 | 6 | 28.65 | 20 | 16.95 | 34 | 7.05 | 48 |
| 41.65 | 7 | 27.75 | 21 | 16.15 | 35 | 6.50 | 49 |
| 40.60 | 8 | 26.85 | 22 | 15.35 | 36 | 5.90 | 50 |
| 39.50 | 9 | 26.00 | 23 | 14.60 | 37 | 5.35 | 51 |
| 38.40 | 10 | 25.10 | 24 | 13.80 | 38 | 4.75 | 52 |
| 37.40 | 11 | 24.20 | 25 | 13.05 | 39 | 4.20 | 53 |
| 36.40 | 12 | 23.40 | 26 | 12.30 | 40 | 3.60 | 54 |
| 35.40 | 13 | 22.60 | 27 | 11.50 | 41 | 3.05 | 55 |
| 34.40 | 14 | 21.75 | 28 | 10.90 | 42 | 2.60 | 56 |
| 33.40 | 15 | 21.00 | 29 | 10.20 | 43 | 2.15 | 57 |
| 32.45 | 16 | 20.15 | 30 | 9.50 | 44 | 1.65 | 58 |
| 31.50 | 17 | 19.35 | 31 | 8.80 | 45 | 1.20 | 59 |
| 30.55 | 18 | 18.55 | 32 | 8.20 | 46 | 0.75 | 60 |

The unreduced copper and hydroxylamine react as follows:

$$4\,CuO + 2\,NH_2OH = 2\,Cu_2O + N_2O + 3\,H_2O.$$

The $Cu_2O$, which is thus formed, is immediately precipitated as white cuprous thiocyanate $Cu_2(SCN)_2$. The hydroxylamine solution is added until the blue color, due to the excess of unreduced copper, just disappears. The following solutions are employed:

(*A*) 250 gms. of pure potassium carbonate, 50 gms. of pure potassium bicarbonate and 200 gms. of potassium thiocyanate are dissolved by warming in about 600 c.c. of water. The liquid is cooled and a cold solution of 12.5 gms. crystallized copper sulphate in about 75 c.c. of water slowly added. The solution is made up to 1000 c.c. and, after standing 24 hours, filtered.

(*B*) 6.55 gms. of pure hydroxylamine sulphate and 200 gms. of potassium thiocyanate are dissolved to 2000 c.c.

One cubic centimeter of *B* should correspond to exactly 1 c.c. of *A*.

* Biochem. Zeitschr., **2**, 271.

In making the determination 10 c.c. of the sugar solution, which should not contain over 60 mgs. of glucose, are measured into a 200-c.c. flask and 50 c.c. of solution *A* added. The liquid is heated to boiling and kept at ebullition for exactly 3 minutes. The solution in then cooled and solution *B* added from a burette until the blue color just disappears. Table LXXIX gives the milligrams of glucose corresponding to the cubic centimeters of hydroxylamine solution used.

**Kendall's Alkaline-salicylate Method.** — Kendall* has recently devised a method for determining reducing sugars, in which salicylic acid and potassium bicarbonate are used in place of the ordinary alkaline-tartrate mixture of Fehling's solution. The advantages claimed are that the alkaline-salicylate mixture has no copper-reducing power of its own and that much larger amounts of copper are reduced by a given weight of sugar when the carbonates of the alkalies are used in place of the hydroxides.

The sugar solution is measured into a 200-c.c. Erlenmeyer flask, and the volume made up to 100 c.c. with distilled water. There are then added in succession 5 gms. salicylic acid, 15 c.c. copper-sulphate solution, containing 133.33 gms. $CuSO_4.5\,H_2O$ per liter, and 25 c.c. potassium-carbonate solution, containing 600 gms. $K_2CO_3$ per liter. The flask is shaken until the salicylic acid has completely dissolved, and then placed in a boiling-water bath for exactly 20 minutes; the reduced cuprous oxide is then filtered upon asbestos, washed with hot water, and the copper determined by Kendall's modified iodide method (p. 412). From the milligrams of copper thus found the corresponding weights of glucose, invert sugar, lactose hydrate and maltose hydrate are determined from a specially calculated table.

### Volumetric-reduction Methods by Means of Mercury Solutions

Of other metallic salt solutions besides copper only those of mercury have been used to any great extent for determining reducing sugars.

**Knapp's† Alkaline Mercuric-cyanide Method.** — The solution used in Knapp's method is prepared by dissolving 10 gms. of pure mercuric cyanide and 100 c.c. of sodium-hydroxide solution of 1.145 sp. gr. to 1000 c.c. The solution contains 7.9363 gms. of metallic mercury per liter.

In making the determination a measured volume of the reagent, previously standardized against a known weight of the pure sugar, is heated to boiling and the sugar solution added from a burette until a drop of the filtered solution shows upon acidifying with acetic acid no coloration with ammonium-sulphide solution. The calculation of

* J. Am. Chem. Soc., **34**, 317. † Z. analyt. Chem., **9**, 395.

sugar is made in the same manner as described under Soxhlet's volumetric method with Fehling's solution.

The end reaction in Knapp's method has been found uncertain and the process at present is but little used.

**Sachsse's* Alkaline Mercuric-iodide Method.** — The solution of Sachsse is prepared as follows: 18 gms. of pure dry mercuric iodide (prepared by precipitating mercuric-chloride solution with potassium iodide, and washing and drying at 100° C.) are dissolved in a solution containing 25 gms. of pure potassium iodide; a solution containing 80 gms. of potassium hydroxide is then added and the volume completed to 1000 c.c. The solution contains 7.9323 gms. of metallic mercury per liter.

An alkaline stannous-chloride solution, prepared by treating a solution of stannous chloride with an excess of potassium hydroxide, is used for determining the end point.

In making the determination a measured volume of reagent is heated to boiling, and the sugar solution added until a drop of the filtered solution shows no coloration with the alkaline tin solution. The comparative reducing power of several sugars upon Sachsse's solution is given in Table LXXXII, page 474.

## Estimation of Higher Saccharides by Determining the Copper-Reducing Power After Hydrolysis

The methods previously described in this chapter for determining reducing sugars are equally applicable to the analysis of the higher non-reducing saccharides provided the latter first undergo a quantitative hydrolysis into sugars of known reducing power.

The best examples of such applications of the method are the determinations of sucrose, starch, dextrin and glycogen by means of Fehling's solution.

### Determination of Sucrose by Means of Fehling's Solution

Sucrose upon treatment with invertase or acids is hydrolyzed quantitatively, 95 parts of sucrose yielding 100 parts of invert sugar. If the copper-reducing power of an inverted-sucrose solution be determined, the equivalent of invert sugar multiplied by the factor 0.95 will give the amount of sucrose present.

In making the determination care must be taken that the amount of sugar after inversion does not exceed the limit of the tables, which for 50 c.c. of mixed Fehling's solution is about 240 mgs. of invert sugar,

* Z. Ver. Deut. Zuckerind., **26**, 872.

or the equivalent of about 225 mgs. of sucrose. The chemist should check the method with pure sucrose, in which case the following procedure may be followed.

Dissolve 1.9 gms. of pure sucrose in about 75 c.c. of water in a 500-c.c. graduated flask and invert the solution according to the method of Herzfeld, or any of the processes described in Chapter X. After cooling, the solution is nearly neutralized with sodium hydroxide (*carefully avoiding any excess*) and the volume completed to 500 c.c.; 50 c.c. of this solution (containing 200 mgs. invert sugar = 190 mgs. sucrose) are then treated according to any of the copper-reduction methods for invert sugar and the weight of reduced copper determined. The milligrams of invert sugar, corresponding to this weight of copper, multiplied by the factor 0.95 gives the milligrams of sucrose.

In applying the method to the determination of sucrose in sugar-house products, and other substances, which contain invert sugar, the difference between the invert-sugar equivalents before and after inversion is multiplied by 0.95. The same methods for determining invert sugar should be employed in both cases. The method of calculation is best illustrated by an example:

Four grams of apple must were made up to 100 c.c. (solution A). Four gms. of the same must were inverted, nearly neutralized and made up to 100 c.c. (solution B).

50 c.c. of sol. B gave by Meissl's method 407 mgs. Cu = 230 mgs. invert sugar
50 c.c. of sol. A gave by Meissl's method 235 mgs. Cu = 126 mgs. invert sugar

Difference = 172 mgs. Cu 104 mgs. invert sugar.

104 mgs. invert sugar $\times$ 0.95 = 98.8 mgs. or 4.94 per cent sucrose.

The mistake is sometimes made of taking the difference between the weights of reduced copper before and after inversion and calculating the invert sugar and sucrose from this. The extent of this error, which is due to the variation in the copper-reducing power for different parts of the table (as shown in Table LXXI), may be seen from the previous example, where a difference of 172 mgs. of copper was found. 172 mgs. of copper according to Meissl's table correspond to 90.8 mgs. of invert sugar. $90.8 \times 0.95 = 86.2$ mgs. or 4.31 per cent of sucrose, a result considerably less than that obtained by the other method.

In calculating sucrose by any of the chemical methods, the reducing sugars before inversion must always be expressed as invert sugar, although it may actually exist as glucose, lactose, maltose, etc., or a mixture of several of these. This, of course, applies only to the sucrose calculation and not to that of the reducing sugars.

*Example.* — 5 gms. of a sirup containing sucrose and maltose were made up to 500 c.c. (solution A). 5 gms. of the same sirup were dissolved, inverted, nearly neutralized and made up to 500 c.c. (solution B).

| | Copper. | Invert sugar. | Maltose. |
|---|---|---|---|
| | Mgs. | Mgs. | Mgs. |
| 50 c.c. of sol. B gave by Munson and Walker's method | 390 | = 215.0 | |
| 50 c.c. of sol. A gave by Munson and Walker's method | 199 | = 103.7 | = 175.5 |
| Difference | 191 | 111.3. | |

$111.3 \times 0.95 = 105.7$ mgs. = 21.14 per cent sucrose in sirup.

175.5 mgs. = 35.10 per cent maltose in sirup.

Calculating the sucrose from the difference in copper, as is sometimes wrongly done, would give the following: 191 mgs. Cu = 99.3 mgs. invert sugar (by Munson and Walker's table), $99.3 \times 0.95 = 94.3$ mgs. = 18.86 per cent sucrose in sirup.

The unified methods and tables are most convenient for converting the equivalents of any reducing sugar into that of invert sugar. The same result, however, may be accomplished by means of the copper-reducing ratios given on page 421.

*Example.* — 10 gms. of a sirup containing sucrose and fructose were made up to 500 c.c. (solution A). 10 gms. of the same sirup were dissolved, inverted, nearly neutralized and made up to 500 c.c. (solution B).

25 c.c. of sol. B gave by Allihn's method 414 mgs. Cu = 221 mgs. glucose

25 c.c. of sol. A gave by Allihn's method 195 mgs. Cu = 100 mgs. glucose

Difference = 121 mgs. glucose.

The reducing ratio of invert sugar to glucose is 0.958 for Allihn's method. $121 \div 0.958 = 126.3$ mgs. invert sugar. $126.3 \times 0.95 = 120$ mgs. = 24.00 per cent sucrose in sirup.

The reducing ratio of fructose to glucose is 0.915 for Allihn's method. $100 \div 0.915 = 109.3$ mgs. = 21.86 per cent fructose in sirup.

Owing to the slight variation in the reducing ratios of some of the sugars, as maltose and lactose, it is more accurate to determine the equivalents by one of the unified methods.

### DETERMINATION OF STARCH BY MEANS OF FEHLING'S SOLUTION

Starch upon heating with dilute hydrochloric acid is hydrolyzed almost quantitatively according to the equation $(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$, in which 90 parts of starch yield 100 parts of glucose. The conversion of starch into glucose may be accomplished either by direct acid hydrolysis, as in Sachsse's method, or by first converting the starch into soluble products, as with diastase, and then hydrolyzing the filtered solution with acid.

**Method of Sachsse, as modified by the Association of Official Agricultural Chemists.*** — Stir a convenient quantity of the sample (representing from 2.5 to 3 gms. of the dry material) in a beaker with 50 c.c. of cold water for an hour. Transfer to a filter and wash with 250 c.c. of cold water. Heat the insoluble residue for two and a half hours with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1.125) in a flask provided with a reflux condenser. Cool, and nearly neutralize with sodium hydroxide; complete the volume to 250 c.c., filter and determine the glucose in an aliquot of the filtrate by any of the usual methods of copper reduction. The weight of glucose multiplied by 0.90 gives the weight of starch.

Owing to the fact that a perfect theoretical yield of glucose is never obtained from starch by acid hydrolysis, Ost † recommends the use of the factor 0.925 for converting glucose into starch by Sachsse's method.

Sachsse's method is one of the simplest processes for estimating starch, but has the objection of converting pentosans and other hemicelluloses into reducing sugars. The method for this reason gives too high results in the analysis of starchy substances which contain much cellular tissue. In order to eliminate this error the starch must be dissolved from cellular substances before hydrolyzing with acid; solution of starch may be effected by heating under pressure or by the action of diastase.

**Method of Determining Starch by Solution under Pressure.‡** — Three grams of the finely-ground sample are extracted with cold water, as in the previous method in order to remove sugars, dextrin, gums, etc. If much oil or fat is present the material should first be extracted with ether. The residue is then heated in a covered flask or metal beaker, of about 200-c.c. capacity, with 100 c.c. of water in an autoclave, a form of which designed by Soxhlet is shown in Fig. 175. The heating is continued for 3 to 4 hours at 3 atmospheres pressure. If an autoclave is not available, Lintner pressure bottles (Fig. 176) may be used; the bottles are immersed in a glycerol bath and heated for 8 hours at 108° to 109° C.

When the digestion is finished the pressure is first allowed to subside, when the autoclave, or pressure flask, is opened and the solution filtered through asbestos. The insoluble residue is well washed with hot water, and should show no blue reaction with iodine when ex-

* Bull. 107 (revised), U. S. Bur. of Chem., p. 53.
† Chem. Ztg., **19,** 1501.
‡ König's "Untersuchung" (1898), p. 221.

amined under the microscope. The filtrate is made up to 200 c.c. and then heated with 20 c.c. of hydrochloric acid, of 1.125 sp. gr., for 3

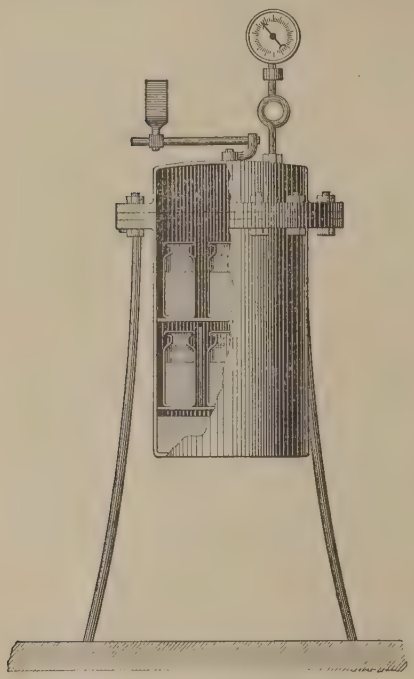


Fig. 175. — Soxhlet's autoclave.

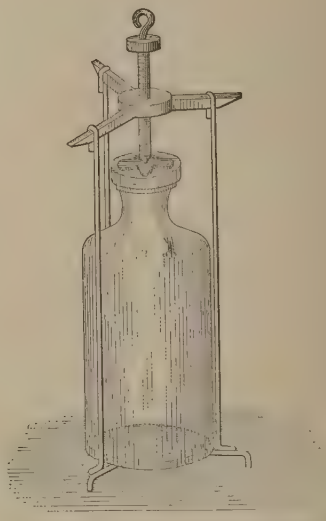


Fig. 176. — Lintner's pressure bottle.

hours in a boiling-water bath, the flask, which holds the solution, being connected with a reflux condenser. The solution, after cooling, is nearly neutralized with sodium hydroxide and made up to 500 c.c. The copper-reducing power of the solution is then determined; the glucose equivalent of the copper multiplied by 0.9 gives the corresponding equivalent of starch.

**Method of Determining Starch by Solution with Diastase.** — Märcker* found that the best method of dissolving starch from hemicelluloses was by means of diastase. The method of Märcker, as modified by the Association of Official Agricultural Chemists, is as follows:

*Preparation of Malt Extract.* — Digest 10 gms. of fresh, finely-ground malt 2 or 3 hours at ordinary temperature with 200 c.c. of

* "Handbuch der Spiritusfabrikation" (1886), 94.

water and filter. Determine the amount of glucose in a given quantity of the filtrate after boiling with acid, etc., as in the starch determination, and make the proper correction in the subsequent determination.

*Determination.* — Extract a convenient quantity of the substance (ground to an impalpable powder and representing from 4 to 5 gms. of the dry material) on a hardened filter with 5 successive portions of 10 c.c. of ether; wash with 150 c.c. of 10 per cent alcohol and then with a little strong alcohol. Place the residue in a beaker with 50 c.c. of water, immerse the beaker in a boiling-water bath and stir constantly for 15 minutes or until all the starch is gelatinized; cool to 55° C., add 20 c.c. of malt extract and maintain at this temperature for an hour. Heat again to boiling for a few minutes, cool to 55° C., add 20 c.c. of malt extract and maintain at this temperature for 1 hour or until a microscopic examination of the residue with iodine shows no starch. Cool and make up directly to 250 c.c.; filter. Place 200 c.c. of the filtrate in a flask with 20 c.c. of hydrochloric acid (sp. gr. 1.125); connect with a reflux condenser and heat in a boiling-water bath for two and one-half hours. Cool, nearly neutralize with sodium hydroxide and make up to 500 c.c. Mix the solution well, pour through a dry filter and determine the glucose in an aliquot of the filtrate by any of the usual methods of copper reduction. The weight of glucose multiplied by 0.90 gives the weight of starch.

Wein * has calculated a table for the above methods which gives the milligrams of starch or dextrin corresponding to milligrams of reduced copper as obtained by Allihn's method. The table was constructed by simply multiplying the milligrams of glucose in Allihn's table by the factor 0.9.

Of the various processes for determining starch the diastase method secures the most perfect solution of starch with the least solution of accompanying hemicelluloses. In cases, however, where much cellular matter is present the hot water and malt solution may dissolve a small amount of pentosans, which, by being afterwards hydrolyzed into reducing pentose sugars, introduce a slight error in the determination.

A more serious error than the above consists in the incomplete hydrolysis of starch into glucose. Experiments by W. A. Noyes,† and his coworkers, testing the action of 2.5 per cent hydrochloric acid upon the malt conversion of starch, show a hydrolysis into glucose which is about 97 per cent of the theoretical. A diminished yield of glucose necessitates the use of a conversion factor somewhat greater than 0.9.

* Wein's "Tabellen." † J. Am. Chem. Soc., **26**, 266.

*Modification of Noyes* for Determining Starch by the Diastase Method.* — In the modification recommended by Noyes the filtrate from the malt digestion is treated with one-tenth its volume of hydrochloric acid of sp. gr. 1.125. "After heating for 1 hour in a flask immersed in a boiling-water bath, making allowance for the time required for the solution to attain the temperature of the bath, the solution is cooled, enough sodium hydroxide is added to neutralize 90 per cent of the hydrochloric acid used, the solution made up to a definite volume, filtered on a dry filter, if necessary, and the reducing power determined by Fehling's solution; 100 parts of glucose found in this manner represent 93 parts of starch in the original material."

Noyes emphasizes the importance of each chemist determining for himself with pure glucose the ratio between glucose and copper for the particular solutions and method which he uses.

### DETERMINATION OF DEXTRIN BY MEANS OF FEHLING'S SOLUTION

The principle of the method is the same as that described for starch. In the process described by König † a weighed amount of the dextrin is dissolved in cold water, made up to 1000 c.c. and filtered. Three portions of 200 c.c. each of the filtrate are heated in a boiling-water bath with 20 c.c. of hydrochloric acid of 1.125 sp. gr. for periods of 1, 2 and 3 hours. The solutions are cooled, nearly neutralized with sodium hydroxide and made up to volume so that the solution does not contain over 1 per cent glucose. The glucose is then determined by any of the usual methods, and the highest results of the three experiments taken as the correct value. The weight of glucose multiplied by the factor 0.9 gives the equivalent of dextrin.

If sugars are also present, the glucose equivalent of these must be subtracted from the glucose equivalent after hydrolysis and the difference calculated to dextrin.

The hydrolysis of dextrin by dilute hydrochloric acid was found by W. A. Noyes ‡ and his co-workers to be a little less than 95 per cent complete at the end of 2 hours' heating and the results seemed to indicate that the theoretical yield of glucose could not be obtained even by prolonged heating. The theoretical factor 0.9 for converting glucose to dextrin is no doubt considerably too low for the method of acid hydrolysis.

* J. Am. Chem. Soc., **26,** 266.
† König's "Untersuchung" (1898), p. 215.
‡ J. Am. Chem. Soc., **26,** 266.

DETERMINATION OF GLYCOGEN BY MEANS OF FEHLING'S SOLUTION

**Pflüger's* Glycogen Method.** — The method is based upon the hydrolysis into glucose of the impure glycogen $(C_6H_{10}O_5)_n$, which has previously been precipitated from the solution of animal substance.

One hundred grams of the finely divided tissue are heated with 100 c.c. of 60 per cent potassium-hydroxide solution, in a boiling-water bath for 3 hours, the flask, which contains the solution, being shaken at frequent intervals. The cooled solution is made up to 400 c.c. and treated with 800 c.c. of 96 per cent alcohol. After standing 24 hours the clear solution is decanted through a filter, the precipitate of impure glycogen stirred with an excess of 60 per cent alcohol and again set aside. The settling of the glycogen in the numerous treatments may be hastened by adding a few drops of concentrated salt solution. The clear liquid is again decanted and the process repeated for a third time. The purification is then continued in the same way, twice with 96 per cent alcohol, once with absolute alcohol, three times with ether and once again with absolute alcohol. Any material adhering to the filter is then removed to the main portion of precipitate, and the raw glycogen dissolved in hot water. The solution is then neutralized with hydrochloric acid of 1.19 sp. gr., and transferred to a 500-c.c. flask; 25 c.c. of hydrochloric acid (sp. gr. 1.19) are then added and the liquid heated in a boiling-water bath for 3 hours. The solution is then cooled, neutralized, made up to 500 c.c., filtered and the glucose determined in the filtrate by Pflüger's method. The amount of glucose multiplied by the factor 0.927 gives the corresponding amount of glycogen.

## Extraction of Sugars and Preparation of Solutions for Chemical Methods of Analysis

The methods and precautions previously given for the extraction of sugars and preparation of solutions for polarimetric examination hold also for the chemical methods of analysis.

**Clarification of Solutions.** — With products which contain but little insoluble matter, such as sugars, molasses, sirups, jellies, honeys, etc., the weighed amount of material is dissolved in water, clarified, if necessary, with a minimum of neutral lead-acetate solution, made up to volume and filtered. The filtrate, after deleading by means of sodium carbonate, sodium sulphate, potassium oxalate or other means, as described on page 276, is then ready for analysis.

With products of high purity, which contain but little mineral matter or organic non-sugars, the use of lead acetate may be dispensed

* Pflüger's Archiv, **114**, 242.

with, and a few cubic centimeters of alumina cream be used for clarification.

*Precipitation of Reducing Sugars by Basic-lead Salts.* — Lead subacetate, or other basic salts of lead, which are employed as clarifying agents in the polarimetric determination of sucrose, should never be used upon solutions in which reducing sugars are to be determined. The action of such compounds in causing a precipitation, or occlusion, of reducing sugars in the lead precipitate has already been mentioned. Bryan * found that basic-lead salts, in presence of magnesium sulphate and ammonium tartrate, precipitated in case of glucose from 3 per cent to 17 per cent, and in case of fructose from 8 per cent to 35 per cent, of the total amount of sugar in solution. Neutral lead acetate under the same conditions caused the precipitation of only 0.9 per cent of the total glucose and 0.0 per cent of the total fructose. (See Table XL, p. 216.)

In a series of independent experiments made by Bryan and Horne † upon raw cane sugar and cane molasses the following results were obtained.

TABLE LXXX

*Showing Influence of Clarification with Lead Subacetate upon Determination of Reducing Sugars*

| | Clarifying agent and analyst. | Allihn's method. | | | Munson and Walker's Method. | | |
|---|---|---|---|---|---|---|---|
| | | Weighing as $Cu_2O$. | Weighing as CuO. | Titration of Cu by Low's Method. | Weighing as $Cu_2O$. | Weighing as CuO. | Titration of Cu by Low's method. |
| | | Per cent | Per cent | Per cent | Per cent | Per cent | Per cent |
| Cane sugar. | *No Clarifying Agent* — | | | | | | |
| | A. H. Bryan | 6.45 | 6.22 | 5.88 | 6.29 | 5.98 | 5.83 |
| | W. D. Horne | 7.08 | 7.05 | 7.02 | 6.43 | 6.51 | 6.37 |
| | Average | 6.77 | 6.63 | 6.45 | 6.36 | 6.25 | 6.10 |
| | *Lead-subacetate Solution* — | | | | | | |
| | A. H. Bryan | 6.14 | 5.67 | 5.67 | 5.76 | 5.51 | 5.30 |
| | W. D. Horne | 6.61 | 6.51 | 6.51 | 6.19 | 6.01 | 5.99 |
| | Average | 6.38 | 6.09 | 6.09 | 5.98 | 5.76 | 5.65 |
| Cane molasses. | *No Clarifying Agent* — | | | | | | |
| | A. H. Bryan | 19.77 | 19.37 | 19.45 | 19.20 | 18.34 | 18.43 |
| | W. D. Horne | 20.60 | 20.06 | 19.97 | 20.00 | 19.43 | 19.44 |
| | Average | 20.19 | 19.72 | 19.71 | 19.60 | 18.89 | 18.94 |
| | *Lead-subacetate Solution* — | | | | | | |
| | A. H. Bryan | 17.51 | 16.47 | 16.29 | 17.27 | 16.26 | 15.97 |
| | W. D. Horne | 19.45 | 19.16 | 19.16 | 19.00 | 18.53 | 18.26 |
| | Average | 18.48 | 17.82 | 17.73 | 18.14 | 17.39 | 17.12 |

* Bull. 116, U. S. Bur. of Chem., p. 73. † Bull. 116, U. S. Bur. of Chem., pp. 72, 74.

Clarification with lead subacetate caused a loss of about 10 per cent of the total reducing sugars present. The variable results, due to method of estimating copper, show a contamination of the cuprous oxide as explained on page 416. The higher results by Allihn's method are due to the greater inverting action of the more strongly alkaline Fehling's solution.

### PREPARATION OF SUGAR SOLUTIONS FROM PLANT SUBSTANCES

If the material to be analyzed contains much insoluble matter, as is the case with plant substances containing cellular tissue, the sugars must first be extracted by means of water or alcohol. In the case of grains, cattle-feeds, etc., the following provisional method is used by the Association of Official Agricultural Chemists.*

**Extraction of Sugars with Cold Water.** — Weigh into a flask or bottle, suitable for stirring or shaking, 10 to 20 gms. of the material, depending upon the amount of soluble carbohydrates present. Add 250 c.c. of ice-cold water, less the volume of water present as moisture in the material, and stir or shake for 4 hours. If enzymatic action is feared the extraction should be made at a low temperature, preferably by surrounding the extraction flask with broken ice; or extract at ordinary temperature with 40 to 50 per cent alcohol. If there is present in the material much soluble substance, correction should also be made for the increase in volume due to solution. If necessary for clear filtration, add from 5 to 10 c.c. of alumina cream, just before filtering. The volume of alumina cream to be added must be taken into account in determining the amount of water used for the extraction. After the extraction filter immediately, pouring back upon the filter the first portions of cloudy filtrate until the filtrate is clear. To free from soluble impurities add sufficient normal lead-acetate solution to 200 c.c. of the filtrate to precipitate all impurities, make up to 250 c.c. and filter. Remove the excess of lead by means of anhydrous sodium carbonate or anhydrous sodium sulphate, followed in the latter case by a small amount of anhydrous sodium carbonate, care being taken not to use an excess. Filter again and use the clear filtrate for the determination of reducing sugars.

The extraction of sugars from plant substances by means of cold water is not always trustworthy owing to the action of enzymes upon sucrose, starch and other higher saccharides. The employment of hot

* Bull. 107 (revised), U. S. Bur. of Chem., p. 57.

water is also often unreliable on account of the solution of hemicelluloses, starch and gums.

**Extraction of Sugars with Dilute Alcohol.** — Bryan, Given and Straughn * have recently made experiments upon the extraction of sugars from grains and similar products, using as solvents 50 per cent alcohol and 0.2 per cent sodium-carbonate solution. Both of these solvents inhibit the action of enzymes and were found to give concordant results upon certain classes of products. In many cases, however, the sodium-carbonate extraction gave much higher amounts of reducing sugar after inversion — a result, perhaps, of the solvent action of the alkali upon pentosans and other hemicelluloses. Bryan, Given and Straughn believe that extraction with 50 per cent alcohol, all points considered, is the most reliable method for general sugar work. The method outlined by them is as follows:

*Method of Bryan, Given and Straughn.* — Place 12 gms. of the finely ground substance in a 300-c.c. graduated flask, adding, in case the material is acid, from 1 to 3 gms. of precipitated calcium carbonate. Add 150 c.c. of neutral alcohol of 50 per cent volume strength; mix thoroughly and boil on a hot-water bath for 1 hour, placing a small funnel in the neck of the flask to condense the vapor. Cool and make up to 300 c.c. with neutral 95 per cent alcohol. After mixing and settling transfer 200 c.c. of the clear solution to a distilling flask and distil off the excess of alcohol, which is thus recovered for future use. The liquid residue is evaporated to a volume of 20 to 30 c.c. (but *not* to dryness), and then washed with water into a 100-c.c. graduated flask. The solution is clarified with the necessary amount of neutral lead-acetate solution, and, after standing 15 minutes, made up to 100 c.c. Pass through a folded filter, carefully saving all of the filtrate, to which add enough anhydrous sodium carbonate to precipitate the excess of lead; allow to stand 15 minutes and then pour through an ashless filter. Over 75 c.c. of filtrate should be obtained; 25 c.c. of the clear filtrate (equivalent to 2 gms. of original material) are diluted with 25 c.c. of water and used for the determination of reducing sugars; 50 c.c. of the same filtrate are transferred to a 100-c.c. flask, inverted with 5 c.c. of concentrated hydrochloric acid, neutralized and made up to 100 c.c. Filter, if necessary, and take 50 c.c. (equivalent to 2 gms. of original material) for the determination of reducing sugars after inversion. The percentages of invert sugar and sucrose are calculated in the usual way and the results multiplied by the factor 0.97 to correct for the volume of insoluble matter.

* Circular 71, U. S. Bur. of Chem.

### PREPARATION OF SUGAR SOLUTIONS FROM ANIMAL SUBSTANCES

**Clarification.** — Liquids of animal origin, such as blood, serum, urine, milk, secretions, extracts, etc., frequently contain large amounts of albuminoids and other nitrogenous substances which interfere with the determination of reducing sugars by the methods of copper reduction. The clarifying agent which is most used for such liquids is mercuric nitrate.

*Mercuric-nitrate Solution.* — Treat 220 gms. of yellow oxide of mercury with 300 to 400 c.c. of water; then add nitric acid in small portions, with warming and stirring, until the precipitate is dissolved. Dilute to 1000 c.c. and filter.

The liquid to be clarified is treated with mercuric-nitrate solution until no more precipitate forms; the solution is then nearly neutralized with sodium-hydroxide solution of 1.3 sp. gr., made up to volume and filtered. A measured portion of the slightly acid filtrate is then freed from excess of mercury by precipitating with hydrogen sulphide; the solution is filtered, the hydrogen sulphide removed by a current of air and the reducing sugars determined by any of the usual methods.

*Clarification of Milk.* — For the clarification of milk, the use of copper sulphate and potassium hydroxide will be found more convenient. The following is the official method of the Association of Agricultural Chemists.*

Dilute 25 c.c. of the milk with 400 c.c. of water and add 10 cc. of a solution of copper sulphate of the strength given for Soxhlet's modification of Fehling's solution. Add about 7.5 c.c. of a solution of potassium hydroxide of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxide from one volume of the solution of copper sulphate. Instead of a solution of potassium hydroxide of this strength, 8.8 c.c. of a half-normal solution of sodium hydroxide may be used. After the addition of the alkali solution the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500-c.c. mark, mix and filter through a dry filter. Determine the lactose by any of the usual methods.

In determining reducing sugars in substances of animal origin, the precipitate of cuprous oxide is often badly contaminated with mineral and organic impurities, so that the reduced copper should be determined directly and not by weighing as suboxide or oxide.

* Bull. 107 (revised), U. S. Bur. of Chem., p. 119.

### CONCENTRATION OF SUGAR SOLUTIONS

In working with very dilute solutions, such as contain only a few hundredths of a per cent of sugar, it is often necessary to concentrate the liquid to one-half, one-fifth or one-tenth the original volume before a satisfactory determination of the copper-reducing power can be made. It is exceedingly important in evaporating such solutions that the liquid be kept exactly neutral, otherwise changes may result in the composition of the sugars. Traces of free acid may become sufficiently concentrated towards the end of evaporation to hydrolyze higher saccharides, and traces of free alkali may modify or destroy reducing sugars.

The evaporation of solutions containing reducing sugars must be conducted in vessels which do not give up soluble alkali; the concentration of sugar solutions in glass vessels, unless of perfect resistant non-soluble quality, is for this reason to be avoided. The author has found flasks and basins of tinned copper to be very suitable for concentrating sugar solutions, there being no change in reducing power after diluting and evaporating to the original volume.

If the solution to be concentrated is slightly acid an excess of finely powdered calcium carbonate (alkali free) will prevent the hydrolysis of higher saccharides. If the solution is alkaline, dilute acetic acid is first added to faint acidity, and then an excess of calcium carbonate. When the evaporation is completed, the residue of insoluble matter is removed by filtration.

# CHAPTER XV

## SPECIAL QUANTITATIVE METHODS

The determination of sugars by means of their reducing power upon Fehling's solution, Sachsse's solution or other metallic salt combinations is a general method, and has no value for the selective determination of particular groups of reducing sugars. For such purposes more special processes of analysis must be adopted. The present chapter will describe a number of the best known of such special quantitative methods.

### Determination of Pentoses and Pentosans

**Theory of Method.** — The methods for determining pentoses and pentosans are due to the researches of Tollens,* and his school; they all depend upon the conversion of the pentose sugars into furfural by distilling with hydrochloric acid, according to the principles described on p. 374. The amount of furfural, which distills over, is determined and calculated to pentoses. The yield of furfural does not correspond perfectly to the equation,

$$\underset{\text{100 parts pentose}}{C_5H_{10}O_5} = \underset{\text{64 parts furfural}}{C_5H_4O_2} + 3\,H_2O,$$

being for arabinose about 75 per cent and for xylose about 90 per cent of the theoretical. Yet by making the distillation under carefully controlled conditions, it is possible, by means of formulæ or tables which have been established for different weights of pure pentoses, to make a determination with a very close degree of approximation.

Different reagents have been used for precipitating the furfural in the determination of pentoses. Tollens and Stone first attempted to determine furfural by precipitating with ammonia as furfuramide. An important advance was then made by Tollens, in company with Günther, de Chalmot, Flint and Mann, in using phenylhydrazine for precipitating the furfural. The use of phenylhydrazine was attended, however, with certain inconveniences and was finally abandoned upon the discovery by Councler † of the precipitating action of phloroglucin.

* For a review of the subject see papers by Tollens with bibliography in Abderhalden's "Arbeitsmethoden," 1909, II, 130, and in Papier-Zeitung, 1907, Nos. 56, 60 and 61 (Reprint).

† Chem. Ztg., **17**, 1743; **18**, 966.

The phloroglucin method, as first developed by Tollens and Krüger,* was further improved by Tollens and Rimbach, and finally established in its present form by Tollens and Kröber.†

**Description of the Method.** — The necessary apparatus for making the determination is shown in Fig. 177. From 2 to 5 gms. of substance, according to the richness of the material in pentoses or pentosans, are placed in a 300-c.c. distillation flask with 100 c.c. of hydrochloric acid

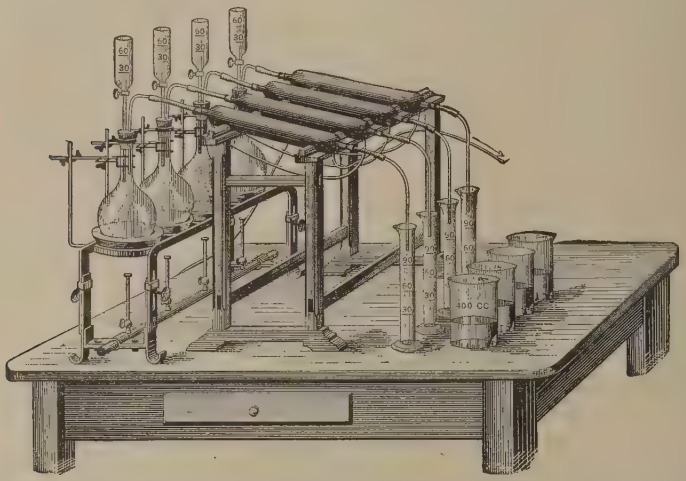



Fig. 177. — Apparatus for determining pentoses and pentosans by distillation with hydrochloric acid.

of 1.06 sp. gr. The flask is closed with a two-hole rubber stopper, one opening of which is fitted to the connecting tube of a condenser and the other to a small separatory funnel. The latter is preferably of cylindrical form with graduation marks at 30 c.c. and 60 c.c. The flask is then placed in a bath of Rose's alloy (1 part lead, 1 part tin and 2 parts bismuth, melting near 100° C.), which, after heating just beyond the point of fusion, is brought up slightly above the level of the bottom of the flask. The distillate is received in a graduated cylinder; when 30 c.c. of liquid have passed over, which should require from 10 to 11 minutes, 30 c.c. more of the hydrochloric acid of 1.06 sp. gr. are added from the separatory funnel. The process is continued in this way until a drop of the distillate shows no pink colora-

* Z. Ver. Deut. Zuckerind., **46**, 21, 195.
† Jour. f. Landwirtsch. (1900), 355, (1901), 7.

tion with aniline-acetate paper (see p. 375). From 9 to 12 portions of 30 c.c. usually require to be distilled over, depending upon the amount of furfural. The distillation is then suspended and the furfural determined by precipitation with phloroglucin.

**Preparation of Phloroglucin.*** — Dissolve a small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling and add a few drops of concentrated sulphuric acid. A violet color indicates the presence of diresorcin. A phloroglucin which gives more than a faint coloration may be purified by the following method:

Heat in a beaker about 300 c.c. of hydrochloric acid (sp. gr., 1.06) and 11 gms. of phloroglucin, added in small quantities at a time, stirring constantly until it has almost entirely dissolved. Some impurities may resist solution, but it is unnecessary to dissolve them. Pour the hot solution into a sufficient quantity of the same hydrochloric acid (cold) to make the volume 1500 c.c. Allow it to stand at least over night — better several days — to allow the diresorcin to crystallize out, and filter immediately before using. The solution may turn yellow, but this does not interfere with its usefulness. In using it, add the volume containing the required amount to the distillate.

**Precipitation of Phloroglucide.** — The distillate obtained by the method previously described is treated in a 500-c.c. lipped beaker with a measured volume of phloroglucin solution, so that the amount of phloroglucin is about double that of the furfural expected. The solution first turns yellow, then green and finally becomes almost black when the amorphous dark-green precipitate of furfural phloroglucide, $C_{11}H_8O_4$, begins to deposit. The liquid is then made up to 400 c.c. with the 12 per cent hydrochloric acid (1.06 sp. gr.) and allowed to stand over night. The solution, after testing with aniline-acetate paper to make sure that all furfural has been precipitated, is filtered through a weighed Gooch crucible; the precipitate of phloroglucide is brought carefully upon the asbestos and washed with 150 c.c. of water in such a way that the water is not entirely removed from the crucible until the very last. The crucible is then placed upon a support, so that the bottom is free to the air, and dried for 4 hours in a boiling-water bath; it is then placed in a weighing bottle, cooled in a desiccator and weighed. The increase in weight is the amount of furfural phloroglucide which is calculated to furfural, pentose or pentosan according to the table of Kröber (Appendix, Table 22).

The weights of pentose in Kröber's table are the averages of the corresponding weights of xylose and arabinose. The weights of pen-

* Bull. 107 (revised), U. S. Bur. of Chem., p. 54.

tosan are obtained by multiplying the corresponding weights of pentose by the factor 0.88, which represents the ratio of $n C_5H_{10}O_5$ to $(C_5H_8O_4)_n$ or $\frac{132}{150}$. The table of Kröber has a range for weights of phloroglucide between 0.030 and 0.300 gms. For weights of phloroglucide outside of these limits Kröber gives the formulæ:

For weight of phloroglucide "$a$" under 0.03 gm.

Furfural = $(a + 0.0052) \times 0.5170$ gm.
Pentoses = $(a + 0.0052) \times 1.0170$ gm.
Pentosans = $(a + 0.0052) \times 0.8949$ gm.

For weight of phloroglucide "$a$" over 0.300 gm.

Furfural = $(a + 0.0052) \times 0.5180$ gm.
Pentoses = $(a + 0.0052) \times 1.0026$ gm.
Pentosans = $(a + 0.0052) \times 0.8824$ gm.

The factor 0.0052 represents the weight (5.2 mgs.) of phloroglucide, which remains dissolved in the 400 c.c. of acid solution.

For weights of phloroglucide which exceed 0.5 gm. it may be found necessary to dry for a longer period than 4 hours in order to attain constancy in weight. It is always better in making the determination to regulate the weight of material so that the amount of phloroglucide falls within the range of the table.

**Precautions and Limitations.** — In making the determination of pentosans by the method of acid distillation, several precautions should be noted. It is important first that the heat be applied to the flask in such a way that charring of solids upon the surface of the glass above the liquid be avoided. Such charring is very apt to occur when the flask is heated over the open flame or upon wire gauze; the use of the metal bath for heating is for this reason to be preferred. It is also important that the distillate be perfectly clear, and free from suspended impurities, before adding the solution of phloroglucin. With substances which contain much oil or wax, fatty decomposition products are sometimes carried over into the distillate; in determining pentoses in the urine of herbivorous animals, benzoic acid (a decomposition product of hippuric acid) is distilled over in considerable amount. In all such cases the distillate must be filtered from suspended matter before precipitating the furfural with phloroglucin.

Two important limitations of the distillation method for determining pentoses should be mentioned. 1. Furfural is formed from other substances than pentoses (the so-called furfuroids). 2. Other substances, which form a precipitate with phloroglucin, are distilled over besides furfural (the so-called furaloids).

"*Furfuroids.*" — The formation of furfural from glucuronic acid and oxycellulose has already been considered (p. 375). The presence of glucuronic acid in urine, or of oxycellulose in plant substances, will introduce, therefore, a certain error in the determination of pentoses in such materials. Cross and Bevan * for this reason propose that the names furfurose, furfurosan or furfuroid be used to designate the furfural-yielding complex of plants. The researches of Tollens show, however, that the pentosans are by far the most important of the furfural-yielding groups; the term pentosans, though not a perfectly correct expression, seems destined to remain until more accurate methods are devised for determining the different furfural-yielding groups.

The distillates obtained by boiling cellulose, starch, sucrose, fructose, glucose and other hexose carbohydrates with hydrochloric acid give with phloroglucin a small yield of phloroglucide corresponding to 0.5 to 1.0 per cent pentosans. Whether the reacting substance in such distillates is furfural, oxymethylfurfural or mixtures of these has not been definitely determined. A slight error is, nevertheless, introduced into the pentose, or pentosan, determination by the phloroglucin method and the chemist should always bear this fact in mind when only small amounts of phloroglucide are obtained.

"*Furaloids.*" — The distillation of other products, which give precipitates with phloroglucin, besides furfural has also been long recognized. Methylfurfural, which is obtained by the distillation of methylpentoses with hydrochloric acid, forms for example a red precipitate with phloroglucin, which, unless removed by solution in alcohol, as afterwards described, will give too high a weight of furfural phloroglucide. In the same way oxymethylfurfural (see p. 620) which is formed in slight amounts by the action of hydrochloric acid upon fructose, sucrose and other hexose carbohydrates, forms a precipitate with phloroglucin.

Fraps † has estimated that the amount of foreign products ("furaloid") in the hydrochloric-acid distillate of different plant substances may vary from 7 to 23 per cent of the crude furfural. The "furaloid" is decomposed according to Fraps by redistilling the acid distillates; the pure furfural thus obtained is precipitated with phloroglucin, the weight of phloroglucide corresponding to the amount of furfural-yielding bodies (pentosans or furfuroids); the difference between the weights of phloroglucide for distillate and redistilled distillate corresponds to the amount of furaloid-yielding bodies, the exact nature of

* Cross and Bevan's "Cellulose" (1895), p. 99.

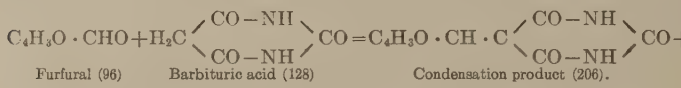
† Am. Chem. Jour., **25**, 501.

which Fraps did not determine. Furaloid does not seem to be formed from the pure pentose sugars.

**Precipitation of Furfural by Means of Barbituric Acid.** — Jäger and Unger * have suggested barbituric acid for precipitating furfural in presence of foreign distillation products. Cellulose, starch, sucrose and other hexose carbohydrates give hydrochloric-acid distillates which, though reacting with phloroglucin, form no precipitate with barbituric acid. Jäger and Unger claim that the reagent offers, therefore, a more accurate means of estimating pentosans.

In making the precipitation the hydrochloric-acid distillate is treated with a solution of pure barbituric acid in hydrochloric acid of 1.06 sp. gr., using 8 parts of barbituric acid to 1 part of estimated furfural. The solution is stirred and after standing 24 hours the yellow granular precipitate filtered into a Gooch crucible, washed with water and dried for 4 hours at 105° C. The weight of precipitate is increased by 0.0049 gm. for the amount of substance dissolved in the 400 c.c. of acid solution.

The reaction between furfural and barbituric acid proceeds as follows:



Furfural (96) Barbituric acid (128) Condensation product (206).

One hundred parts of condensation product thus correspond to 46.6 parts of furfural.

The barbituric-acid method for determining pentosans offers several good features, but the process has not been tried sufficiently as yet by chemists to form a conclusion as to its reliability.

**Jolles's Method of Determinating Pentoses.** — Jolles † has recently proposed a method for determining pentoses which differs in several particulars from that of Tollens. The substance to be distilled is placed in a 1500 c.c. flask with 200 c.c. of 12 per cent hydrochloric acid; the flask is heated, while a current of steam is passed through the liquid, the distillation being regulated so that the volume of solution does not fall at any time below 100 c.c. By distilling the furfural with steam the formation of humus substances is said to be prevented and a quantitative yield of furfural obtained. The process is continued until 1 c.c. of the distillate shows no coloration with Bial's orcin reagent (p. 382); 100 c.c. of the distillate (usually between 2 and 3 liters)

* Ber., **35**, 4440; **36**, 1222.

† Sitzungsber. Wiener Akad., **114** (II b), 1191 (1905).

are neutralized with sodium hydroxide, and then made faintly acid to methyl orange with a few drops of half-normal hydrochloric acid. A measured volume of $\frac{1}{10}$-normal sodium-bisulphite solution is then added, and the solution allowed to stand 2 hours. The amount of bisulphite, remaining after the reaction with the furfural, is then titrated back with $\frac{1}{10}$-normal iodine solution, using starch solution as indicator. The difference between the volumes of bisulphite and iodine solutions gives the amount of bisulphite which entered into combination with the furfural. The reaction between the two is expressed by the equation:

$$C_4H_3O \cdot CHO + NaHSO_3 = C_4H_3O \cdot CH\begin{matrix} OH \\ SO_3Na \end{matrix}$$

The titration of an aliquot, which is less than 5 per cent of the total distillate, involves a very great multiplication of any experimental errors. Jolles's process has not as yet demonstrated its superiority over the much shorter and simpler method of Tollens.

The method of Tollens for determining pentoses gives good results with pure arabinose or xylose but, as has been shown, yields only rough approximations in the case of the various furfuroids. Even in the case of pure pentosans the calculation of furfural to a mixture of araban or xylan in equal amounts, when perhaps the pentosan itself may consist almost entirely of one substance, may involve an error of several per cent in the calculation. In certain plant exudations, as cherry gum, the pentosans consist almost entirely of araban; in the hemicelluloses of certain woods, as the beech, almost entirely of xylan; in the encrusting substances of most cellular tissues of variable mixtures of araban and xylan. Until accurate methods are available for the estimation of xylan and araban, and for the determination of oxycellulose and other furfuroids, the calculation of furfural to a mixture of xylan and araban in equal amounts can be regarded only as a conventional approximation.

**Applications of Pentosan Method.** — The determination of pentosans, notwithstanding certain limitations of the method, has found numerous applications in the assay of plant gums, in the analysis of feeding materials, in the examination of forestry products and in other ways. A single example of such application is given in the analysis of paper stock. Kröber,* for example, gives the following determinations of pentosans in different raw materials used in paper manufacture.

* Jour. f. Landwirtsch. (1901), 7.

TABLE LXXXI

| Material. | Pentosans calculated to ash-free dry substance. |
|---|---|
| | Per cent. |
| Mechanical wood pulp | 12.24 |
| Mechanical wood pulp | 11.93 |
| Cotton | 1.03 |
| Linen | 2.20 |
| Bleached straw | 26.76 |
| Bleached raw cellulose (soda process) | 6.41 |
| Bleached raw cellulose (sulphite process) | 7.09 |

An application of the above results to a special problem, which may confront the paper chemist, is taken from the work of Tollens.*

A sample of newspaper is known to be made up of mechanical wood pulp and sulphite cellulose; it is desired to know the percentages of each which were used. The sample of paper upon analysis showed 10 per cent pentosans calculated to ash-free dry substance. Calling the percentage of pentosans in the ash-free dry substance of mechanical wood pulp 12 per cent and of sulphite cellulose 7 per cent, then

$$\frac{10-7}{12-7} \times 100 = 60 \text{ per cent mechanical wood pulp.}$$

$$\frac{12-10}{12-7} \times 100 = 40 \text{ per cent sulphite cellulose.}$$

For other applications of the method the chemist is referred to the original paper by Tollens.

### DETERMINATION OF METHYLPENTOSES AND METHYLPENTOSANS

The conversion of methylpentoses into methylfurfural by distillation with hydrochloric acid was described on p. 377. The method for determining methylpentoses, or methylpentosans, is based upon determining the amount of methylfurfural which is thus produced. The details of the method, which were first worked out by Tollens and Ellett,† and further elaborated by Tollens and Mayer,‡ are practically the same as described for the determination of the pentoses. The same apparatus (Fig. 177) is used and the substance is distilled with 12 per cent hydrochloric acid (1.06 sp. gr.) until a drop of the distillate gives no yellow coloration with aniline-acetate paper. The methylfurfural is then precipitated with phloroglucin and the solution allowed to remain over night, when the red precipitate of methylfurfural

* Reprint Papier-Zeitung (1907), p. 17.

† Ber., **38**, 492.

‡ Z. Ver. Deut. Zuckerind. (1907), 620; Ber., **40**, 2441.

phloroglucide is filtered, washed, dried and weighed in exactly the same manner as described for furfural phloroglucide.

The weight of methylfurfural phloroglucide is then calculated either to rhamnose by the table of Ellett and Tollens or to fucose by the table of Mayer and Tollens. The rhamnose, $CH_3C_5H_9O_5 \cdot H_2O$, is calculated to rhamnosan $(CH_3C_5H_7O_4)_n$ by multiplying by the factor $\frac{1}{1}\frac{4}{8}\frac{6}{2} = 0.80$; and the fucose, $CH_3C_5H_9O_5$, to fucosan by the factor $\frac{1}{1}\frac{4}{6}\frac{6}{4} = 0.89$. The combined table giving the weights of rhamnose, rhamnosan, fucose, fucosan, and methylpentosan (mixture of equal parts rhamnosan and fucosan) corresponding to different weights of methylfurfural phloroglucid is given in the Appendix (Table 23).

Instead of the tables the following formulæ may be used in which Ph is the weight in grams of methylfurfural phloroglucide.

$$\text{Fucose} = 2.66\ \text{Ph} - 12.25\ \text{Ph}^2 + 0.0005.$$
$$\text{Rhamnose} = 1.65\ \text{Ph} - 1.84\ \text{Ph}^2 + 0.0100.$$
$$\text{Methylpentosan} = 1.85\ \text{Ph} - 6.25\ \text{Ph}^2 + 0.0040.$$

Fucose decomposes slower than rhamnose with hydrochloric acid, so that the distillation must be continued longer. More decomposition products of methylfurfural are consequently formed in distilling fucose with a corresponding less yield of phloroglucide.

Methylfurfural, according to Fromherz,* may also be estimated by precipitation with barbituric acid in the same manner as described for furfural. The reaction takes place according to the equation:

$$CH_3C_4H_2O \cdot CHO + H_2C\begin{matrix} \diagup CO-NH \diagdown \\ \diagdown CO-NH \diagup \end{matrix}CO$$
$$= CH_3C_4H_2O \cdot CH \cdot C\begin{matrix} \diagup CO-NH \diagdown \\ \diagdown CO-NH \diagup \end{matrix}CO + H_2O.$$

Methylfurfural (110) Barbituric acid (128) Condensation product (220)

Two parts of condensation product thus correspond to exactly one part of methylfurfural. The yellow crystalline precipitate is filtered in a Gooch crucible, washed with water and then dried for 5 hours in a steam bath. The precipitate is then weighed, and after correcting for its slight solubility in the 12 per cent hydrochloric acid (2.29 mgs. in 100 c.c.), calculated to methylfurfural by dividing by 2.

According to Jolles † methylfurfural may also be determined by his method of steam distillation and titration with bisulphite and iodine solutions. The reaction between bisulphite and metyhlfurfural is similar to that described for bisulphite and furfural, and the details of the two methods are exactly alike.

* Z. physiol. Chem., **50, 241.**

† Ann., **351, 41.**

### Determination of Pentoses and Methylpentoses in Mixture

**Method of Tollens and Ellett.** — The method of determining pentoses and methylpentoses in mixture was first worked out by Tollens and Ellett,* and is based upon the solubility of methylfurfural phloroglucide, and the insolubility of furfural phloroglucide in warm 95 per cent alcohol.

In making the determination the material is distilled with 12 per cent hydrochloric acid, the distillate precipitated with phloroglucin, and the mixed phloroglucides of furfural and methylfurfural filtered in a Gooch crucible, dried and weighed according to the usual process.

The crucible containing the mixed phloroglucides is then placed in a smaller beaker with 95 per cent alcohol which is heated nearly to boiling. The brown-colored solution is then sucked off through the crucible by means of a filter pump, and the extraction with hot 95 per cent alcohol repeated twice more in the same way. The crucible containing the insoluble furfural phloroglucide is then dried for 2 hours in a hot-water bath and reweighed in a weighing bottle. The residual weight of furfural phloroglucide is then calculated to pentoses or pentosans and the loss in weight, due to methylfurfural phloroglucide, calculated to methylpentoses, or methylpentosans, by means of the respective tables or formulæ.

Trials of this method of separation upon known mixtures of pentoses with methylpentoses were made by Ellett and Tollens, and by Mayer and Tollens with very close agreements.

*Modification by Haywood of the Tollens-Ellett Method.* — Haywood,† who has recently tested the method of Tollens and Ellett, believes that a correction should be made for the slight solubility of the furfural phloroglucide in 95 per cent alcohol. Experiments made by Haywood upon the phloroglucide obtained from pure arabinose showed that for varying weights of substance, and extracting 3 to 5 times with alcohol, a very uniform weight of about 0.0037 gm. was always dissolved. Haywood believes the substance thus dissolved to be occluded phloroglucin and not phloroglucide. The following slight modification of the Tollens-Ellett method is proposed by Haywood:

Place the Gooch crucible containing the mixed phloroglucides in a 100-c.c. beaker and pour into the crucible 30 c.c. of 95 per cent alcohol heated to 60° C. Place the beaker for 10 minutes in a water bath heated to 60° C. Remove the beaker and crucible and suck from the

* Z. Ver. Deut. Zuckerind. (1905), 19.

† Bull. 105, U. S. Bur. of Chem., p. 112.

latter all alcohol remaining therein with a suction pump. Repeat this alternate extraction and sucking dry of the precipitate 3 to 5 times, according to the color of the filtrate obtained. After the final extraction place the Gooch crucible in a water oven and dry four hours, making the final weighing in a closely stoppered glass weighing bottle.

The difference in weight between the furfural phloroglucide plus methylfurfural phloroglucide first obtained and the furfural phloroglucide remaining after extraction with alcohol, minus 0.0037, represents the amount of methylfurfural phloroglucide present, from which the methylpentose or methylpentosan is calculated by the tables or formulæ.

To obtain the weight of pentosans, subtract the corrected weight of methylphloroglucide from the weight of the mixture and calculate according to Kröber's tables or formulæ.

### Determination of Galactose or Galactan

Tollens * and his co-workers have developed a method for estimating galactose, and its higher condensation product galactan $(C_6H_{10}O_5)_n$, which is based upon a determination of the mucic acid formed by oxidation of the substance with nitric acid. The oxidation of galactose to mucic acid according to theory proceeds as follows:

$$\underset{\text{Galactose (180)}}{C_6H_{12}O_6} + 2\,HNO_3 = \underset{\text{Mucic acid (210)}}{C_6H_{10}O_8} + 2\,H_2O + 2\,NO.$$

100 parts of galactose thus equal 116.66 parts of mucic acid. In actual experiment only about 75 per cent of the weight of galactose is obtained as mucic acid. This yield, however, is fairly constant for the given conditions of analysis, so that the weight of mucic acid multiplied by $1\frac{1}{3}$ gives the weight of galactose.

The method of Tollens as employed by the Association of Official Agricultural Chemists † is as follows:

Extract a convenient quantity of the substance, representing from 2.5 to 3 grams of the dry material, on a hardened filter with 5 successive portions of 10 c.c. of ether; place the extracted residue in a beaker about 5.5 cm. in diameter and 7 cm. deep, together with 60 c.c. of nitric acid of 1.15 sp. gr., and evaporate the solution to exactly one-third its volume in a water bath at a temperature of 94° to 96° C. After standing 24 hours, add 10 c.c. of water to the precipitate, and allow it to stand another 24 hours. The mucic acid has in the meantime crystallized but it is mixed with considerable material only par-

* Ann., **227,** 223; **232,** 187.

† Bull. 107 (revised), U. S. Bur. of Chem., p. 55.

tially oxidized by the nitric acid. Filter the solution, therefore, through filter paper, wash with 30 c.c. of water to remove as much of the nitric acid as possible, and replace the filter and contents in the beaker. Add 30 c.c. of ammonium-carbonate solution, consisting of 1 part ammonium carbonate, 19 parts of water and 1 part strong ammonium hydroxide, and heat the mixture on a water bath, at 80° C., for 15 minutes, with constant stirring. The ammonium carbonate takes up the mucic acid, forming the soluble mucate of ammonia. Then wash the filter paper and contents several times with hot water by decantation, passing the washings through a filter paper, to which finally transfer the material and thoroughly wash. Evaporate the filtrate to dryness over a water bath, avoiding unnecessary heating which causes decomposition; add 5 c.c. of nitric acid of 1.15 sp. gr., thoroughly stir the mixture and allow to stand for 30 minutes. The nitric acid decomposes the ammonium mucate, precipitating the mucic acid; collect this on a tared filter or Gooch crucible, wash with from 10 to 15 c.c. of water, then with 60 c.c. of alcohol and a number of times with ether; dry at the temperature of boiling water for 3 hours, and weigh. Multiply mucic acid by 1.33, which gives galactose and multiply this product by 0.9 which gives galactan.

The method of Tollens has been used considerably by Schulze and Steiger * for determining galactan groups in different plants of the Leguminosæ and also by Bauer † for estimating galactose and lactose in the urine.

The presence of large amounts of foreign organic matter hinders the precipitation of mucic acid, and in case of only small amounts of the latter may prevent its separation entirely. The tendency of the method is, therefore, to give too low rather than too high results.

### Fermentation Methods for Determining Sugars

A method for estimating sugars has been described (p. 299) which is based upon the change in polarization which the solution undergoes after fermenting with yeast.

The fermentation methods for determining sugars are more usually carried out by weighing or measuring the carbon dioxide which is evolved. The theoretical yield of carbon dioxide from glucose, according to the equation $C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$, is 48.88 per cent. In actual experiments only about 45 per cent of $CO_2$ is obtained, this figure varying, however, by several per cent according to the variety

* Landw. Vers. Stat., **36,** 11; **36,** 438, 465.

† Z. physiol. Chem., **51,** 159.

of yeast, influence of non-sugars and other conditions. The weight of carbon dioxide obtained during a normal fermentation multiplied by the factor 2.2 will give the approximate amount of fermentable hexose sugars present. The fermentation method is employed almost entirely for determining small percentages of sugar, and has found its widest application in the determination of glucose in urine.

**Direct Method by Weighing Carbon Dioxide.**—The most accurate method for determining the yield of carbon dioxide upon fermentation

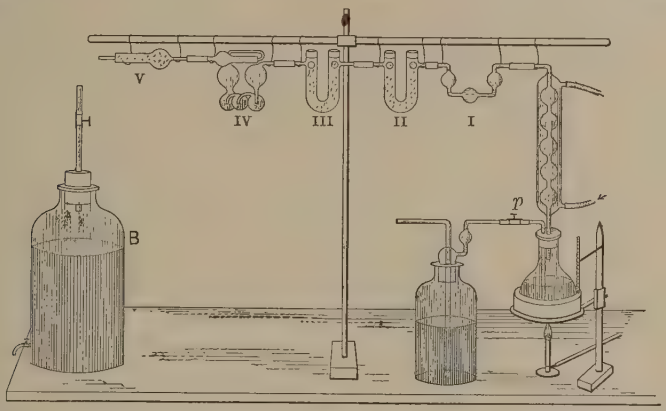



Fig. 178.—Apparatus for determining sugars from weight of carbon dioxide given off by fermentation.

is shown in Fig. 178. A known amount of the solution is sterilized in a small flask, then cooled and inoculated with a pure culture of yeast. The flask is then connected by means of a condenser with a train of absorption tubes, or bulbs. Bulb I (Fig. 178) contains a few cubic centimeters of water, the U-tubes II and III contain calcium chloride for removing all moisture from the current of gas, the Liebig potash bulb IV, which has been previously weighed, serves to absorb the carbon dioxide, and the safety tube V, containing calcium chloride and soda lime, prevents back absorption of water, or carbon dioxide, from the outside air. The fermentation is allowed to proceed either at room temperature, or, if desired, at 30° C., in which case the flask is immersed in a water bath carefully maintained at this temperature. At the end of 1 to 2 days, when no more gas passes through the bulb I, the tube V is connected with the aspirator bottle *B*, the pinchcock at *p*, which is previously closed, opened and a slow current of air, freed from carbon dioxide by passing through potassium hydroxide solution, led through

the apparatus. At the end of an hour the liquid in the flask is heated nearly to boiling, while a current of cold water circulates through the condenser; in this manner the last traces of dissolved carbon dioxide are expelled from the liquid. The aspiration is continued for another hour, when the potash bulb IV is disconnected and reweighed. The increase in weight gives the amount of carbonic acid.

The more usual process, in the fermentation method of estimating sugars, is to estimate the carbon dioxide by measuring the volume of gas; 1 c.c. of evolved carbon dioxide (at 0° C. and 760-mm. atmospheric pressure) corresponds to 1.96 mgs. carbon dioxide or about 4 mgs. of glucose. For determining sugars by this method special forms of apparatus known as fermentation saccharometers have been devised, of which the two forms devised by Einhorn and by Lohnstein are selected as examples.

**Einhorn's Fermentation Saccharometer.*** — This apparatus, which is designed for the estimation of small amounts of glucose in diabetic urine, is shown in Fig. 179. One gram of commercial pressed yeast is shaken thoroughly in the graduated test tube with 10 c.c. of the urine. The mixture is then poured into the bulb of the saccharometer, the apparatus being inclined so that the graduated tube is completely filled. The saccharometer is then set aside for 20 to 24 hours at ordinary temperature. If the urine contains sugar, fermentation will usually begin in about 30 minutes. When the fermentation is finished the volume of gas is measured in the graduated tube, the divisions of which indicate cubic centimeters of gas and also the approximate fractions of per cent glucose. If the urine contains more than 1 per cent glucose it must first be diluted with water, the reading of the saccharometer being then multiplied by the degree of dilution. For diabetic urines of straw color and a specific gravity of 1.018 to 1.022 it is recommended to dilute twice; of 1.022 to 1.028 sp. gr. 5 times, and 1.028 to 1.038 sp. gr. 10 times.

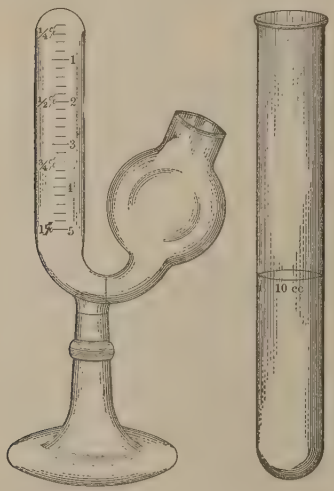



Fig. 179. — Einhorn's fermentation saccharometer.

* Circular of information.

It is always desirable in making the test to make a duplicate determination upon a normal urine. The latter should show at most only a small bubble of gas at the top of the tube; should a larger amount of carbon dioxide be obtained with normal sugar-free urine, the yeast is probably impure and the determination should be repeated. If the suspected urine shows no more gas than the control experiment the absence of glucose is indicated.

**Lohnstein's* Fermentation Saccharometer.** — In Lohnstein's saccharometer (Fig. 180) the liquid is fermented over mercury in a closed bulb; the carbon dioxide, which is evolved, forces the mercury into an upright tube, the amount of displacement indicating the per cent of glucose present.

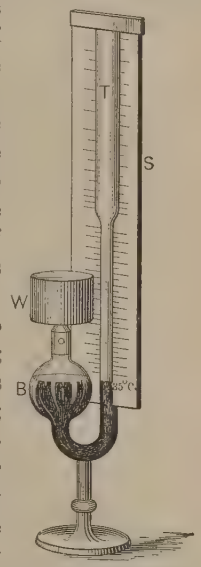


Fig. 180.—Lohnstein's fermentation saccharometer.

In making a determination the detachable scale *S* is hung in position over the open end of the tube *T*, and a quantity of mercury poured into the bulb *B* until its level in the tube is just opposite the zero mark of the scale. The standard weight of mercury, necessary for the adjustment, accompanies each instrument.

A small piece of pressed yeast is rubbed with 2 to 3 times its volume of ordinary water to a thin paste; 0.5 c.c. of the urine, or other liquid to be tested, is then measured with a special pipette into the bulb; the pipette is rinsed into the bulb with a little ordinary water and 2 to 4 drops of the yeast water added. The glass stopper, which should be evenly greased, is then inserted, and turned so that the small opening on its inner surface comes directly opposite a similar opening in the stem of the bulb. Any pressure of air, due to inserting the stopper, is thus released. The stopper is again slightly turned, so as to seal the contents of the bulb hermetically, and then securely fastened by the weight *W*. The apparatus is then set aside until fermentation is finished, which is indicated by the stationary position of the mercury column. The length of time necessary for completing the test will depend upon the temperature but does not ordinarily exceed 1 day at 20° C.; if an incubator is available the time may be shortened considerably by fermenting at 35° C. When fermentation is finished the scale division opposite the top of the mercury column indicates the

* Münchener med. Wochenschr. (1899), No. 50; also circular of information.

percentage of sugar; for percentages of sugar below 2.0 the scale may be read to 0.01 per cent and for percentages between 2.0 and 10.0 to 0.05 per cent. The scale is calibrated upon one side for 20° C. and upon the other for 35° C.; if the readings be made at intermediary temperatures the percentage of sugar is calculated by interpolating. Thus:

The reading of the mercury column at 25° C. was 4.0 on the 20° C. scale and 3.6 on the 35° C. scale. The corrected percentage of sugar is then $3.6 + \frac{4.0 - 3.6}{35 - 20}(35 - 25) = 3.87$ per cent.

Instead of finding the weight or volume of carbon dioxide the percentage of fermentable sugar may also be calculated from the amount of alcohol which is found by the action of yeast, or from the difference in specific gravity of the solution before and after fermentation. A valuable check upon the accuracy of the results obtained by the fermentation methods is to determine the loss in reducing sugars by means of Fehling's solution.

### Colorimetric Methods for Determining Sugars

A number of colorimetric methods have been devised for determining small amounts of different sugars in solution. The first process of this kind was due to Dubrunfaut who determined small percentages of glucose by comparing the color, which was produced by heating the solution with alkalies, with the colors of solutions containing known amounts of pure glucose, which had been similarly treated.

In addition to the alkalies many of the special reagents, used in making color and spectral reactions, such as $\alpha$-naphthol, resorcin, etc., have been employed for the colorimetric estimation of sugars. The principal requirement in the use of such reagents for quantitative purposes is that the color produced must be perfectly soluble and of a fair degree of stability. The insoluble, or evanescent, colors, which are produced in many of the reactions for sugars, are valueless for colorimetry.

For making accurate comparisons of intensity of color, a special apparatus, called a colorimeter, must be used. The colorimeter of Duboscq is one of the best known and is selected for description.

**Duboscq's* Colorimeter.** — The colorimeter of Duboscq, as modified by Pellin, is shown in Fig. 181. The apparatus consists of an upright case, the front and sides of which are in one piece $B$, and hinged to the back. At the bottom of the case is a shelf $S$, containing

* Circular of information.

two circular openings, above which rest the two cylinders $C$ and $C'$. The latter are very carefully constructed, being closed at the bottom by disks of glass whose upper and lower surfaces are perfectly plane parallel. Two immersion rods of solid glass, $T$ and $T'$ — the ends of

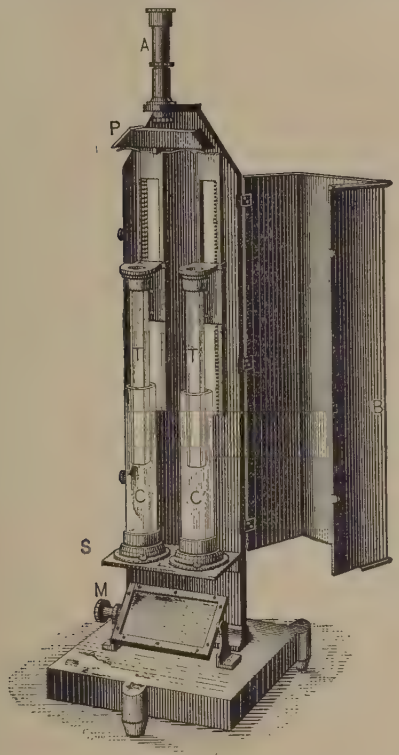



Fig. 181.

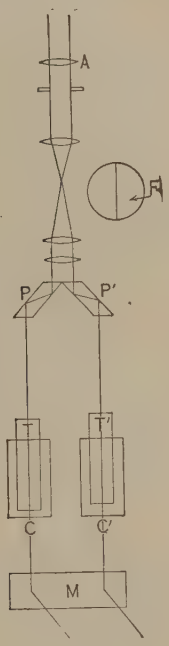



Fig. 182.

Duboscq's colorimeter.

which are also plane parallel — are attached to movable slides in the back of the case and can be raised or lowered within the cylinders. The height of the lower surface of each rod above the bottom of its cylinder is indicated upon a scale, which by means of a vernier can be read to 0.1 mm. The colorimeter is illuminated by light from the reflector $M$, which from its opposite surfaces gives either bright or diffused light according to the requirements of sensibility. The light, as shown in Fig. 182, passes upward through each cylinder and immersion rod to

the prisms $P$ and $P'$, from which it is reflected upwards into the telescope $A$. The field, when the telescope is focused, consists of a circle $F$, divided into equal parts, exactly resembling the double field of a polariscope. Daylight is to be preferred for illuminating the colorimeter although artificial white, or monochromatic, light may be used according to requirement. In preparing the instrument for use, the mirror must be adjusted so that both halves of the field appear of exactly equal intensity.

The sugar solution which is to be tested is placed in one cylinder and the standard solution, containing a known percentage of the same sugar, in the other, both solutions having been previously treated under similar conditions with alkali or other color-producing reagent. The door of the case is then closed and the rod immersed in the solution to be tested to some convenient scale division, as 100 mm., 50 mm., etc., at which point the color of its half of the field should be of suitable intensity for comparison. The other rod is then immersed in the cylinder of standard solution, and lowered or raised until the two halves of the field are of equal intensity. The heights of the immersion rods above the bottoms of the cylinders will then be inversely proportional to the depth of color and hence to the amount of sugar in solution. The calculation is made as follows:

If $A$ = the elevation of rod in standard solution,
$B$ = the elevation of rod in solution to be tested,
$P$ = the per cent of sugar in standard solution,
$X$ = the per cent of sugar in solution to be tested,

then $X = \frac{A \times P}{B}$.

*Example.* — 50 gms. of a glucose solution of unknown strength were made up to 500 c.c. with water, adding 5 c.c. of dilute NaOH solution (solution I).

One gram of pure glucose was dissolved in water and the solution made up to 500 c.c. adding also 5 c.c. of the same NaOH solution (solution II).

Both solutions were heated in a hot-water bath for the same length of time and after cooling compared in a Duboscq colorimeter.

When the immersion rod in solution I was set at 100 mm., the immersion rod in solution II gave equal intensity to the field at 160.2 mm.

Then $\frac{160.2 \times 1}{100} = 1.60$ gms. of glucose in the 500 c.c. of solution I, or 3.2 per cent in the original sample.

Johnson* has recommended heating with alkaline picric-acid solution for the colorimetric determination of glucose. Picric acid is reduced

* Mon. scient., III, **13**, 939.

by glucose and other sugars in alkaline solution to picramic acid, the deep red color of which is sharply developed by less than 0.01 per cent of sugar. As stable color standards Johnson recommends solutions of ferric acetate, or of ferric chloride and acetic acid, which have been prepared so as to match the color produced by a known weight of sugar under the conditions of the method.

Many of the color reactions of sugars are affected by the presence of organic or mineral impurities; the usefulness of colorimetric methods in estimating sugars is for this reason largely curtailed.

**Ehrlich's Colorimetric Method for Estimating Caramel.** — Ehrlich* has devised a colorimetric method for estimating caramel, in which the standard of comparison is saccharan. This dark-colored caramel substance is produced by heating sucrose in a flask immersed in oil to about 200° C. under vacuum. The residue, after extracting with boiling methyl alcohol, is dissolved in water, filtered and evaporated. The saccharan, $C_{12}H_{18}O_9$, is obtained as a dark-brown residue (about 20 per cent of the weight of sucrose) which is easily pulverized to an amorphous powder. One part of saccharan in 10,000 of water colors the solution a deep brown, which is intensified by the addition of alkalies. Saccharan is not precipitated by lead sub-acetate solution, so if the latter is used for precipitating other coloring substances from solutions of sugars, molasses, etc., the percentage of saccharan in the neutralized filtrates may be estimated by comparison in a colorimeter with a solution containing a known weight of saccharan. The amount of saccharan multiplied by 5 indicates the approximate amount of sucrose destroyed by superheating during manufacture.

**Stammer's Colorimeter.** — Colorimeters are employed in technical sugar analysis for grading sirups, for estimating the decolorizing power of bone black or other clarifying agent, and for many other purposes in which degree of color, and not determination of color-producing substance, is desired. For determinations of this kind colored plates, or disks, of glass are usually employed as a standard of comparison, the results being expressed in units of an arbitrary color scale.

A colorimeter which is used extensively in the sugar industry is that of Stammer† (Fig. 183). The general principle of this apparatus is the same as that of Duboscq. The liquid to be tested is placed in the cylinder *a*, which is closed by a glass plate at the bottom. The measuring tube *c*, also closed at the bottom by a glass plate, fits

* Z. Ver. Deut. Zuckerind., **59,** 746. Proceedings, Seventh International Congress of Applied Chem., Sect., V, p. 92.

† Stammer's "Zuckerfabrikation" (1887), p. 747.

loosely into *a* and can be raised or lowered to any desired level. The comparison tube *b*, which is open at the bottom, is joined to *c*, the two being moved in conjunction by a slide in the back of the instrument. The colorimeter is illuminated by a reflector at the bottom, the light passing upward through *b* and *c* into the prisms in *d* which produce the same double-field effect as in the Duboscq apparatus.

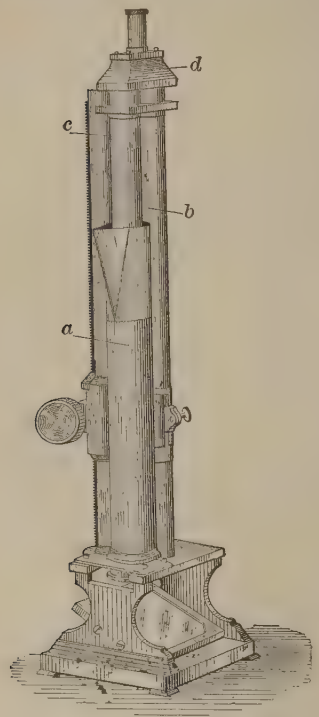



Fig. 183. — Stammer's colorimeter.

In operating the colorimeter the standard plate of colored glass is placed upon tube *b*, which together with tube *c* is then raised or lowered until the intensity of shade for solution and color plate is the same in both halves of the field. A millimeter scale upon the back of the instrument marks the elevation of the measuring tube above the bottom of the cylinder, thus indicating the thickness of the column of liquid.

Stammer gives a solution which matches the standard plate for a scale reading of 1 mm., a color value of 100. The color value of any liquid is found by dividing 100 by the reading of the scale in millimeters.

In measuring the color of sugars, molasses, etc., a weighed amount of substance is dissolved in water, made up to a definite volume and, if the solution is not clear, filtered. The color value of the solution is then calculated either to the original amount of substance, or to a polarization of 100, according to requirement.

*Example.* — 20 gms. of a sugar, polarizing 92.4, were dissolved to 100 c.c. and filtered. The solution gave a reading of 15 mm. upon Stammer's colorimeter. Then $\frac{100}{15} = 6.666$ the color value of the solution. The color value calculated to 100 parts sugar would be $20 : 6.666 :: 100 : x = 33.33$. The latter calculated to 100 polarization would give $92.4 : 33.33 :: 100 : x = 36.07$.

For determining the decolorization produced by bone black the color value of the solution is taken before and after filtration. If the

original solution is too dark for reading in the colorimeter, it is diluted with water, in which case the filtered solution is also diluted to the same density.

*Example.* — An unfiltered sirup diluted to 10 degrees Brix gave a reading of 8 mm., or $\frac{100}{8} = 12.5$ color units, using a Stammer colorimeter. The liquid, after filtering through bone black, and diluting to 10 degrees Brix gave a reading of 40 mm., or $\frac{100}{40} = 2.5$ color units. The amount of color removed by the bone black is then $\frac{12.5 - 2.5}{12.5} \times 100 = 80$ per cent.

A table of reciprocals (Appendix, Table 25) will be found convenient for converting the scale measurements of Stammer's colorimeter into color units.

### Determination of Sugars by Weighing as Hydrazones and Osazones

The varying solubility of the different hydrazones and osazones of sugars in presence of impurities, or of other similar derivatives, has prevented the general employment for quantitative purposes of this means of separating sugars. In certain cases, however, where the hydrazone, or osazone, is characterized by great insolubility a fairly accurate determination of several of the sugars has been found possible.

**Determination of Arabinose as Diphenylhydrazone.** — According to Neuberg * arabinose is precipitated quantitatively by treating the sirupy solution of sugar with a slight excess of diphenylhydrazine. Sufficient alcohol is added to form a perfectly clear solution, and the mixture heated to boiling for 30 minutes in a water bath in a flask connected with a reflux condenser. The solution is cooled, allowed to stand for several hours and the white crystalline hydrazone filtered into a weighed Gooch crucible. After washing with a few cubic centimeters of cold alcohol, the crucible is dried in a water oven and weighed.

The weight of arabinose diphenylhydrazone, $C_5H_{10}O_4N \cdot N(C_6H_5)_2$, is calculated to arabinose, $C_5H_{10}O_5$, by multiplying by $\frac{150}{318} = 0.4747$. This method of analysis has been used by Neuberg for estimating arabinose in the urine and by Maurenbrecher and Tollens † for determining arabinose in cacao.

**Determination of Mannose as Phenylhydrazone.** — The property of mannose in forming with phenylhydrazine a very insoluble hydrazone, discovered by Fischer and Hirschberger,‡ has been used for the quantitative estimation of mannose. The precipitation, according to

* Ber., **35,** 2243. † Ber., **39,** 3578. ‡ Ber., **21,** 1805.

Bourquelot and Herissey,* is best accomplished by treating a 3 to 6 per cent solution of the sugar with an excess of phenylhydrazine acetate at a temperature not above 10° C. After standing 24 hours, the white crystalline hydrazone is filtered upon a weighed Gooch crucible, washed with a little cold water, dried in a water oven and weighed. The solubility of the hydrazone is 0.04 gm. in 100 c.c. of solution, and the weight of precipitate should be corrected accordingly.

The weight of mannose phenylhydrazone, $C_6H_{12}O_5N_2HC_6H_5$, is calculated to mannose, $C_6H_{12}O_6$, by multiplying by $\frac{180}{270} = \frac{2}{3}$, or 0.6666. The method is well adapted for determining mannose in presence of other sugars and has been employed by Pellet † for estimating small amounts of mannose in sugar-cane molasses.

**Determination of Fructose as Methylphenylosazone.** — According to Neuberg ‡ fructose may be determined with a fair approximation by precipitating as its methylphenylosazone, $C_6H_{10}O_4(N_2CH_3C_6H_5)_2$. About 10 c.c. of the concentrated sugar solution are treated with a slight excess of methylphenylhydrazine, and sufficient alcohol added to give a clear solution. If other sugars than fructose are present the solution is slightly warmed and allowed to stand 24 hours for the separation of any insoluble hydrazones of mannose, galactose, etc. After removing any precipitate by suction, the filtrate is treated with 4 c.c. of 50 per cent acetic acid, heated 5 to 10 minutes upon the water bath, and then set aside in the cold for 24 hours. The reddish-yellow crystals of the osazone are filtered in a weighed Gooch crucible and calculated to fructose, $C_6H_{12}O_6$, by multiplying by $\frac{180}{386} = 0.4663$. The method is only approximate as 10 per cent or more of the osazone remains in solution. By using a very cold freezing mixture the separation has been made almost quantitatively.

### Sieben's Method for Estimating Fructose

Sieben § in 1884 proposed a method for determining fructose which is based upon the destruction of this sugar when heated with dilute hydrochloric acid. The method was designed for estimating fructose in honey, sirups and other products which contain glucose. The latter sugar, like other aldoses, is much less susceptible to the destructive action of acids, so that the difference in the reducing power of a solu-

* Compt. rend., **129,** 339.
† Bull. assoc. chim. sucr. dist., **16,** 1181; **18,** 758.
‡ Ber., **35,** 960.
§ Z. Ver. Deut. Zuckerind. (1884), 837, 865.

tion before and after treatment by Sieben's process is taken as the equivalent of the fructose present.

In making the determination 100 c.c. of the solution, which should contain about 2.5 gms. of total reducing sugars, are heated in a 250-c.c. graduated flask with 60 c.c of 6-normal hydrochloric acid ($36.47 \times 6 = 218.8$ gms. HCl per liter) for 3 hours in a boiling-water bath. A funnel is placed in the neck of the flask to prevent evaporation. The solution is then cooled and neutralized with 6-normal sodium hydroxide ($40 \times 6 = 240$ gms. NaOH per liter), of which from 56 to 58 c.c. are usually required. The contents of the flask are then made up to 250 c.c., filtered and the reducing sugars determined in 25 c.c. of the filtrate by Allihn's method. The reducing sugar thus found is calculated as glucose, and the difference in reducing sugar before and after the acid treatment estimated as fructose.

According to Sieben only about 1.5 per cent of the total glucose is destroyed under the conditions of his method. Herzfeld * found, however, that the destruction of glucose may exceed 7 per cent. Wiechmann † also showed that the complete destruction of the fructose is not always assured so that "the results obtained by this method must be received with some caution." Dammüller ‡ found that the destructive power of the acid depended largely upon the ratio of glucose to fructose; with mixtures of glucose and fructose in equal proportions only 1.28 per cent of glucose was destroyed, with pure glucose on the other hand the loss exceeded 28 per cent. Attempts to modify and improve the process so as to overcome these objections have not been wholly successful.

* Z. Ver. Deut. Zuckerind., **35,** 967.
† Wiechmann's "Sugar Analysis" (1898), **p. 54.**
‡ Z. Ver. Deut. Zuckerind., **38,** 751.

# CHAPTER XVI

## COMBINED METHODS AND THE ANALYSIS OF SUGAR MIXTURES

In previous chapters upon polariscopic and chemical methods several instances were given of the application of certain processes to the analysis of sugar mixtures. In the present chapter the problem of determining several sugars in presence of one another will be taken up in somewhat fuller detail.

If the sum of the specific rotations, copper-reducing powers or other properties of the different sugars in a mixture can be expressed by a sufficient number of equations, the problem of determining the percentage of each sugar in the mixture may be solved by simple algebraic analysis. By thus combining the results of several distinct methods it is possible by indirect means to make an analysis of many sugar mixtures with a fair degree of accuracy. The combinations of methods, which have been proposed for this purpose, are almost numberless and only a few examples will be chosen to illustrate the general principle. The methods will be grouped for convenience under (1) Combined polariscopic methods; (2) Combined reduction methods; (3) Combined polariscopic and reduction methods.

### Combined Polariscopic Methods

If two sugars, $A$ and $B$, exhibit a known variation in specific rotation under different conditions of polarization, then the percentages, $x$ and $y$, of the two sugars may be determined by means of the following equations:

$$ax + by = 100[\alpha]_D, \quad (1)$$

$$a'x + b'y = 100[\alpha]_{D'}, \quad (2)$$

in which $[\alpha]_D$ and $[\alpha]_D'$ are the specific rotations of the mixture $A + B$, $a$ and $a'$ the known specific rotations of sugar $A$ and $b$ and $b'$ the known specific rotations of sugar $B$, under the respective conditions of (1) and (2). By determining $[\alpha]_D$ and $[\alpha]_D'$, the percentages $x$ and $y$ are readily calculated.

As an example of this method of analysis the determination of glucose and fructose by polarization at 20° C. and 87° C., under the

conditions previously described (p. 296), is given. If the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of glucose are $+52.5$ and of fructose $-92.5$ and $-52.5$ respectively, then the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of a mixture containing $x$ per cent glucose and $y$ per cent fructose are

$$52.5\,x - 92.5\,y = 100[\alpha]_D^{20}$$
$$52.5\,x - 52.5\,y = 100[\alpha]_D^{87}$$

By determining the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of the mixture the percentages of glucose and fructose are readily calculated.

Any other temperature, at which the $[\alpha]_D^t$ of each of the sugars is known, may of course be taken instead of 20° C. and 87° C. The results as thus calculated are of course only approximate and require to be corrected for the influence of concentration.

In addition to varying the temperature, changes of condition may be accomplished by making one polarization in neutral and the other in acid solution; or one polarization in water, and the other in some other solvent; or one polarization in the absence and the other in the presence of borax or other substance; in all of which changes of condition a definite known alteration in the polarizing power of one or both sugars must be produced. Obviously the greater the degree of this change in polarizing power, the less will be the influence of experimental errors.

### Combined Reduction Methods

If two sugars, $A$ and $B$, exhibit a known variation in reducing power under different conditions of analysis, then the percentages $x$ and $y$ of the two sugars may be determined by means of the general equations:

$$ax + by = 100\,R, \qquad (1)$$
$$a'x + b'y = 100\,R', \qquad (2)$$

in which $R$ and $R'$ are the reducing powers of the mixture $A + B$, $a$ and $a'$ the known reducing powers of sugar $A$, and $b$ and $b'$ the known reducing powers of sugar $B$, under the respective conditions of (1) and (2). By determining $R$ and $R'$, the percentages $x$ and $y$ are readily calculated.

A good example of the application of the above formulæ is given by Soxhlet's * well-known method for determining two sugars in mixture.

A comparison of the reducing powers of different sugars upon Fehling's copper solution (Soxhlet's formula) and Sachsse's mercury solution was made by Soxhlet with the following results:

* J. prakt. Chem. (1880), **21**, 300; König's "Untersuchung" (1898), 217.

TABLE LXXXII

*Showing Relative Reducing Power of Fehling's and Sachsse's Solutions*

| Sugar. | 1 gm. sugar in 1 per cent solution reduces | | Milligrams of sugar in 1 per cent solution reduce | |
|---|---|---|---|---|
| | Fehling's solution. | Sachsse's solution. | 100 c.c. Fehling's solution. | 100 c.c. Sachsse's solution. |
| | c.c. | c.c. | Mgs. | Mgs. |
| Glucose | 210.4 | 302.5 | 475.3 | 330.5 |
| Fructose | 194.4 | 449.5 | 514.4 | 222.5 |
| Invert sugar | 202.4 | 376.0 | 494.1 | 266.0 |
| Galactose | 196.0 | 226.0 | 510.2 | 442.0 |
| Milk sugar | 148.0 | 214.5 | 675.7 | 466.0 |
| Milk sugar hydrolyzed | 202.4 | 257.7 | 494.1 | 388.0 |
| Maltose | 128.4 | 197.6 | 778.8 | 506.0 |

The results show that the various sugars differ very decidedly in their relative reducing powers upon the two reagents, glucose, for example, reducing more Fehling's but less Sachsse's solution than fructose.

The combined influences of two sugars, $A$ and $B$, in their reducing powers upon Fehling's and Sachsse's solutions may be expressed as follows:

Let $x$ = gms. of reducing sugar $A$ in 100 c.c. of the 1 per cent sugar solution.

Let $y$ = gms. of reducing sugar $B$ in 100 c.c. of the 1 per cent sugar solution.

Let $a$ = c.c. of Fehling's solution reduced by 1 gm. of sugar $A$ in 100 c.c. of solution.

Let $b$ = c.c. of Fehling's solution reduced by 1 gm. of sugar $B$ in 100 c.c. of solution.

Let $a'$ = c.c. of Sachsse's solution reduced by 1 gm. of sugar $A$ in 100 c.c. of solution.

Let $b'$ = c.c. of Sachsse's solution reduced by 1 gm. of sugar $B$ in 100 c.c. of solution.

Let $F$ = c.c. of Fehling's solution reduced by 100 c.c. of sugar solution.

Let $S$ = c.c. of Sachsse's solution reduced by 100 c.c. of sugar solution.

Then $$ax + by = F,$$

and $$a'x + b'y = S.$$

For a mixture of $x$ per cent glucose and $y$ per cent fructose, and taking Soxhlet's values in Table LXXXII for $a$, $b$, $a'$ and $b'$, the equations would be

$$210.4\,x + 194.4\,y = F$$
$$302.5\,x + 449.5\,y = S.$$

By determining the values $F$ and $S$ of the mixture of sugars, the percentages $x$ and $y$ are readily calculated.

In using the above, or other combined reduction methods, the constants $a$, $b$, $a'$ and $b'$ should be determined empirically by the chemist for the particular sugars with which he is working.

As another example of combined reduction methods may be mentioned Kjeldahl's* process of determining the reducing power of the mixture of two sugars in both dilute and more concentrated solution, using respectively 15 c.c. and 50 c.c. of mixed Fehling's solution according to the details of his reduction method (p. 424). The relative differences in the copper-reducing powers under the two conditions of analysis are not sufficiently pronounced, however, to afford a reliable basis of calculation and the method has been generally condemned.

The use of combined polariscopic, or of combined reduction, methods alone for analyzing sugar mixtures has largely given place to the more accurate procedure of combining these two distinct physical and chemical methods in one.

## Combined Polariscopic and Reduction Methods

### 1. Analysis of mixtures containing two sugars

The calculation of the percentages of two sugars in mixture by combining the results of polarization and copper reduction was first attempted by Neubauer† in 1877, and the principle of his indirect method has been that of most subsequent modifications. In the earlier methods of this class the total reducing power of the mixture was determined as glucose, fructose or invert sugar, the percentage thus obtained being taken as the total amount, or sum, of the sugars present. In the case of two sugars, $A$ and $B$, the percentages $x$ and $y$ of each were expressed by the formula

$$x + y = R$$

in which $R$ was the percentage of total reducing sugar determined as glucose, fructose or invert sugar. The results calculated by such a formula have, however, only an approximate value, as the difference in copper-reducing power of the two sugars $A$ and $B$ has not been taken into account.

The error last mentioned has been largely obviated in the later methods of this class through the use of reduction factors (p. 421) by means of which the copper-reducing power of a sugar can be converted into the equivalent of any other reducing sugar which is selected as a standard of comparison. For the latter purpose glucose is usually

* Z. analyt. Chem., **35**, 345–347. † Ber., **10**, 827.

selected, this being the most common of the reducing sugars and the one most easily obtained in a pure condition.

It was shown upon p. 421 that the different monosaccharides bear a constant ratio to glucose for the same weight of reduced copper. This ratio was given for several sugars and was found by Allihn's method to be 0.915 for fructose, 0.958 for invert sugar, 0.898 for galactose, 0.983 for xylose and 1.032 for arabinose.

For a solution containing a mixture of monosaccharides, the sum of the glucose equivalents of the individual sugars should equal the total reducing sugars estimated as glucose. This is shown in the following experiments by Browne,* who mixed known weights of different sugars and compared the calculated glucose equivalents with the amount of glucose corresponding to the reduced copper obtained by Allihn's method.

TABLE LXXXIII

*Showing Glucose Equivalents of Mixed Reducing Sugars*

| Sugars. | Grams sugar in 25 c.c. | | | Total weight of sugars. | Glucose equivalent. | | Error. |
|---|---|---|---|---|---|---|---|
| | 1. | 2. | 3. | | Calculated. | Found. | |
| | | | | Gram. | Gram. | Gram. | |
| Glucose, fructose | 0.0967 | 0.0904 | ..... | 0.1871 | 0.1794 | 0.1780 | +0.0014 |
| " " | 0.0484 | 0.0452 | ..... | 0.0936 | 0.0898 | 0.0906 | −0.0008 |
| " " | 0.0461 | 0.1408 | ..... | 0.1869 | 0.1749 | 0.1755 | −0.0006 |
| " " | 0.0231 | 0.0704 | ..... | 0.0935 | 0.0875 | 0.0877 | −0.0002 |
| " " | 0.0740 | 0.0198 | ..... | 0.0938 | 0.0921 | 0.0927 | −0.0006 |
| " galactose | 0.1786 | 0.0585 | ..... | 0.2371 | 0.2311 | 0.2294 | +0.0017 |
| " " | 0.0893 | 0.0293 | ..... | 0.1186 | 0.1156 | 0.1161 | −0.0005 |
| " " | 0.0265 | 0.0960 | ..... | 0.1225 | 0.1127 | 0.1132 | −0.0005 |
| Fructose, galactose | 0.0681 | 0.0175 | ..... | 0.0856 | 0.0780 | 0.0764 | +0.0016 |
| " " | 0.0155 | 0.1070 | ..... | 0.1225 | 0.1102 | 0.1097 | +0.0005 |
| " arabinose | 0.1853 | 0.0569 | ..... | 0.2422 | 0.2282 | 0.2267 | +0.0015 |
| " " | 0.0927 | 0.0285 | ..... | 0.1212 | 0.1141 | 0.1131 | +0.0010 |
| Galactose, xylose | 0.2162 | 0.0429 | ..... | 0.2591 | 0.2361 | 0.2369 | −0.0008 |
| " " | 0.1081 | 0.0215 | ..... | 0.1296 | 0.1181 | 0.1183 | −0.0002 |
| Xylose, arabinose | 0.1513 | 0.0433 | ..... | 0.1946 | 0.1934 | 0.1933 | +0.0001 |
| " " | 0.0757 | 0.0217 | ..... | 0.0974 | 0.0967 | 0.0981 | −0.0014 |
| " " | 0.0495 | 0.1535 | ..... | 0.2030 | 0.2070 | 0.2083 | −0.0013 |
| " " | 0.0248 | 0.0768 | ..... | 0.1016 | 0.1035 | 0.1044 | −0.0009 |
| Glucose, arabinose, xylose | 0.1371 | 0.0226 | 0.0609 | 0.2206 | 0.2203 | 0.2210 | −0.0007 |
| Glucose, galactose, fructose | 0.0646 | 0.0822 | 0.0967 | 0.2435 | 0.2270 | 0.2280 | −0.0010 |

The weights in columns 1, 2 and 3 are given in the order of the respective sugars as named.

The calculated glucose equivalents of the mixtures were found by multiplying the weights of each sugar by its reducing ratio and adding together the products.

The greatest difference between the calculated glucose equivalents and those determined by experiment is 0.0017 gm., which is within the limits of experimental error. It seems, therefore, safe to conclude that

* J. Am. Chem. Soc., **28**, 443.

the reducing ratio of a sugar remains the same whether it occurs alone or with other monosaccharides.

**General Formulæ for Analysis of Sugar Mixtures.** — If the reducing ratio of sugar $A$ to glucose is $a$, and of sugar $B$ to glucose $b$, then in a mixture of $x$ per cent $A$ and $y$ per cent $B$, the combined influence is represented by the equation:

$$ax + by = R \tag{1}$$

in which $R$ is the percentage of total sugars determined as glucose.

If the relative polarizing power of sugar $A$ be expressed by $\alpha$ and that of sugar $B$ by $\beta$, then in a mixture of $x$ per cent $A$ and $y$ per cent $B$, the combined influence is represented by the equation:

$$\alpha x + \beta y = P \tag{2}$$

in which $P$ is the polarizing power of the mixture of sugars.

By combining equations (1) and (2) we obtain:

$$x = \frac{bP - \beta R}{\alpha b - a\beta}. \tag{3}$$

$$y = \frac{\alpha R - aP}{\alpha b - a\beta}, \text{ or } \frac{R - ax}{b}. \tag{4}$$

When the constants $a$, $b$, $\alpha$ and $\beta$ are known, the percentages $x$ and $y$ of any two monosaccharides can be calculated very closely from the percentage of total reducing sugar, determined as glucose, and from the polarizing power of the mixture.

**Applications of the Method.*** — In the following applications of the preceding formulæ to special problems of analysis, the polarizations were made upon a Ventzke-scale saccharimeter using the sucrose normal weight. The relative polarizing power of a sugar under these conditions is best expressed in terms of sucrose and is found by dividing its specific rotation by the specific rotation of sucrose, or +66.5.

In making up the various mixtures the sugars were weighed in a small stoppered flask. After adding the requisite amount of water the flask was reweighed and the percentage of each sugar in the solution calculated. After the sugars were dissolved, the solutions were allowed to stand 24 hours before beginning the analysis, in order to remove all possibility of error through mutarotation.

*Analysis of Mixtures of Fructose and Glucose.* —

Reducing ratio of fructose to glucose = 0.915 = $a$.

Reducing ratio of glucose to glucose = 1.000 = $b$.

* The applications of the method to the analysis of mixtures containing two sugars are taken from the paper by Browne upon "The Analysis of Sugar Mixtures," J. Am. Chem. Soc., **28**, 439.

Polarizing ratio of fructose (20° C., 10 per cent solution) to sucrose

$$= \frac{-90.18}{+66.5} = -1.356 = \alpha.$$

Polarizing ratio of glucose (10 per cent solution) to sucrose

$$= \frac{+52.74}{+66.5} = 0.793 = \beta.$$

By substituting the values for $a$, $b$, $\alpha$ and $\beta$ in the general equations previously given, we obtain:

$$\text{Per cent fructose } (F) = \frac{0.793\,R - P}{2.08} = 0.381\,R - 0.481\,P, \text{ at } 20^\circ \text{ C.} \quad (1)$$

$$\text{Per cent glucose} = R - 0.915\,F. \quad (2)$$

Owing to the great susceptibility of fructose to variations in specific rotation through changes of temperature and concentration, the use of a fixed polarization factor is only possible when the analyses are made under perfectly similar conditions. The values of the polarization factor of fructose for different temperatures and concentrations are given below:

| Temperature. Deg. C. | Concentration. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 per cent. | 2 per cent. | 3 per cent. | 4 per cent. | 5 per cent. | 10 per cent. | 25 per cent. |
| 15 | −1.384 | −1.385 | −1.387 | −1.389 | −1.390 | −1.398 | −1.422 |
| 20 | −1.341 | −1.343 | −1.345 | −1.346 | −1.348 | −1.356 | −1.380 |
| 25 | −1.299 | −1.301 | −1.303 | −1.304 | −1.306 | −1.314 | −1.338 |
| 30 | −1.257 | −1.259 | −1.261 | −1.262 | −1.264 | −1.272 | −1.296 |

The above figures were calculated from the general formula of Jungfleisch and Grimbert, $[\alpha]'_D = -(101.38 - 0.56\,t + 0.108\,(c - 10))$.

The variations of the polarization constant due to concentration are so small that they do not affect the accuracy of the calculations appreciably and a 10 per cent concentration was taken as the basis. The influence of temperature, however, is so pronounced that it cannot be disregarded.

For other temperatures than 20° C. the denominator in equation (1) for fructose becomes 2.12 at 15° C., 2.04 at 25° C. and 2.00 at 30° C.

The percentage of invert sugar in mixtures of glucose and fructose is easily found by combining the smaller percentage with an equal amount of the other component. Thus, in the first experiment of the following series there would be 1.96 per cent invert sugar and 1.13 per cent glucose, and in the last experiment 7.52 per cent invert sugar and 7.47 per cent fructose.

The following analyses were made of seven mixtures containing known amounts of fructose and glucose:

| Taken. | | *R* | *P* | Temp. °C. | Found. | | Error. | |
|---|---|---|---|---|---|---|---|---|
| Fructose. Per cent. | Glucose. Per cent. | | | | Fructose. Per cent. | Glucose. Per cent. | Fructose. Per cent. | Glucose. Per cent. |
| 0.99 | 2.06 | 3.01 | + 0.35 | 22° | 0.98 | 2.11 | −0.01 | +0.05 |
| 1.59 | 5.92 | 7.41 | + 2.65 | 23° | 1.56 | 5.98 | −0.03 | +0.06 |
| 3.17 | 11.83 | 14.54 | + 5.30 | 23° | 3.02 | 11.78 | −0.15 | −0.05 |
| 4.52 | 4.84 | 9.06 | − 2.15 | 22° | 4.51 | 4.83 | −0.01 | −0.01 |
| 5.63 | 1.85 | 7.02 | − 6.00 | 23° | 5.61 | 1.89 | −0.02 | +0.04 |
| 9.04 | 9.67 | 17.80 | − 4.30 | 22° | 8.90 | 9.66 | −0.14 | −0 01 |
| 11.26 | 3.69 | 14.04 | −12.00 | 23° | 11.23 | 3.76 | −0.03 | +0.07 |
| | | | | | Average error..... | | −0.06 | ±0.04 |

*Applications of the Method.* — The formulæ for calculating the percentages of glucose and fructose in mixture admit of numerous applications. The determinations of fructose by this means have been found by the author to show usually a very close agreement with the results obtained by the method of high-temperature polarization, when other copper-reducing or optically active substances are absent.

In the determination of fructose and glucose in cider vinegar, Mott* has shown that the presence of copper-reducing aldehydes may introduce a considerable error in the calculation. If the aldehydes, however, are first volatilized by evaporating the vinegar to dryness in a platinum dish, dissolving the solids in water and again evaporating several times, the true copper-reducing power of the mixed sugars is obtained, in which case the results of the calculation agree closely with those obtained by the method of high-temperature polarization. The following table by Mott gives the percentages of fructose and glucose in the dry substance of several cider vinegars as calculated by Browne's formula and the excess of fructose over glucose as thus found and as determined by polarization at 87° C.

| Variety of Vinegar | Computed by formulæ of Browne | | | Excess of fructose over glucose by polarizing at 87° C |
|---|---|---|---|---|
| | Fructose in solids | Glucose in solids | Excess of fructose | |
| | Per cent | Per cent | Per cent | Per cent |
| Baldwin..... | 19.7 | 8.8 | 10.9 | 10.9 |
| King..... | 18.7 | 7.4 | 11.3 | 11.8 |
| Greening..... | 23.1 | 9.1 | 14.0 | 13.9 |
| Russet..... | 16.0 | 8.6 | 7.4 | 7.2 |
| Mixed, pressing.... | 14.2 | 7.1 | 7.1 | 8.6 |

* J. Ind. Eng Chem., **3**, 747.

*Analysis of Mixtures of Glucose and Galactose.* —

Reducing ratio of glucose to glucose = 1.000 = $a$.

Reducing ratio of galactose to glucose = 0.898 = $b$.

Polarizing ratio of glucose (10 per cent solution) to sucrose

$$= \frac{+52.74}{+66.5} = 0.793 = \alpha.$$

Polarizing ratio of galactose (20° C., 10 per cent solution) to sucrose

$$= \frac{+80.49}{+66.5} = 1.21 = \beta.$$

By substituting the values for $a$, $b$, $\alpha$ and $\beta$ in the general equations, we obtain:

$$\text{Per cent glucose } G = \frac{1.21\,R - 0.898\,P}{0.498} = 2.43\,R - 1.803\,P, \text{ at } 20^\circ \text{ C.} \quad (3)$$

$$\text{Per cent galactose} = \frac{R - G}{0.898}. \quad (4)$$

The specific rotation of galactose varies somewhat with temperature and concentration, the differences, however, being much less than those of fructose. The following values for the polarization factor of galactose at different temperatures and concentrations were calculated from the general formula of Meissl.

| Temperature. Degrees. C. | 10 per cent. | 15 per cent. | 20 per cent. |
|---|---|---|---|
| 10 | 1.242 | 1.248 | 1.254 |
| 20 | 1.210 | 1.216 | 1.222 |
| 30 | 1.179 | 1.185 | 1.191 |

The concentration influence of galactose upon the polarization factor is too slight to influence the calculations appreciably; the temperature influence, however, should be regarded in case the readings are made very much above or below 20° C.

The following analyses were made of four mixtures containing known amounts of glucose and galactose. The polarizations were taken at 25° C. at which temperature the per cent glucose $= \frac{1.195\,R - 0.898\,P}{0.482}$.

| Taken. | | $R$ | $P$ | Temp. °C. | Found. | | Error. | |
|---|---|---|---|---|---|---|---|---|
| Glucose. | Galactose. | | | | Glucose. | Galactose. | Glucose. | Galactose. |
| Per cent. | Per cent. | | | | Per cent. | Per cent. | Per cent. | Per cent. |
| 2.12 | 7.68 | 9.06 | +11.0 | 25° | 1.97 | 7.89 | −0.15 | +0.21 |
| 4.24 | 15.35 | 18.16 | +21.9 | 25° | 4.23 | 15.51 | −0.01 | +0.16 |
| 7.15 | 2.34 | 9.29 | + 8.5 | 25° | 7.20 | 2 33 | +0.05 | −0.01 |
| 14.29 | 4.68 | 18.35 | +17.0 | 25° | 13.82 | 5.04 | −0.47 | +0.34 |
| | | | | | Average error..... | | ±0.17 | ±0.18 |

The average error in the above series of experiments is nearly four times that found in the separation of fructose and glucose. This was to be expected since, owing to the small difference in the specific rotations of glucose and galactose, the errors of observation are doubled; in the analysis of the fructose-glucose mixtures on the other hand the wide range in the specific rotation diminishes the experimental errors one-half.

*Analysis of Mixtures of Fructose and Galactose.* —

Reducing ratio of fructose to glucose $= 0.915 = a.$

Reducing ratio of galactose to glucose $= 0.898 = b.$

Polarizing ratio of fructose (20° C., 10 per cent solution) to sucrose

$$= \frac{-90.18}{+66.5} = -1.356 = \alpha.$$

Polarizing ratio of galactose (20° C., 10 per cent solution) to sucrose

$$= \frac{+80.49}{+66.5} = 1.21 = \beta.$$

By substituting the above values for $a$, $b$, $\alpha$ and $\beta$, in the general equations we obtain:

$$\text{Per cent fructose } (F) = \frac{1.21\,R - 0.898\,P}{2.324} = 0.521\,R - 0.386\,P \text{ (20° C.)}. \quad (5)$$

$$\text{Per cent galactose} = \frac{R - 0.915\,F}{0.898} = 1.114\,R - 1.019\,F. \quad (6)$$

The susceptibility of the specific rotations of both fructose and galactose to temperature variations necessitates a considerable correction if the polarizations are made much above or below 20° C. By using the polarization factors for fructose and galactose previously given, formula (5) can be corrected for any desired temperature. Thus for 30° C. per cent fructose $= \frac{1.179\,R - 0.898\,P}{2.221}$.

The following analyses were made of four mixtures containing known amounts of glucose and galactose.

| Taken. | | $R$ | $P$ | Temp. °C. | Found. | | Error. | |
|---|---|---|---|---|---|---|---|---|
| Fructose. | Galactose. | | | | Fructose. | Galactose. | Fructose. | Galactose. |
| Per cent. | Per cent. | | | | Per cent. | Per cent. | Per cent. | Per cent. |
| 1.24 | 8.56 | 8.78 | + 8.75 | 28° | 1.14 | 8.62 | +0.10 | +0.06 |
| 2.47 | 17.12 | 17.78 | +17.40 | 25° | 2.46 | 17.29 | −0.01 | +0.17 |
| 5.44 | 1.40 | 6.11 | − 5.35 | 28° | 5.38 | 1.33 | −0.06 | −0.07 |
| 10.89 | 2.80 | 12.31 | −10.50 | 29° | 10.76 | 2.74 | −0.13 | −0.06 |
| | | | | | Average error..... | | ±0.07 | ±0.09 |

*Analysis of Mixtures of Fructose and Arabinose.* —

Reducing ratio of fructose to glucose = 0.915 = $a$.

Reducing ratio of arabinose to glucose = 1.032 = $b$.

Polarizing ratio of fructose (20° C., 10 per cent solution) to sucrose

$$= \frac{-90.18}{+66.5} = -1.356 = \alpha.$$

Polarizing ratio of arabinose to sucrose $= \frac{+104.5}{+66.5} = 1.571 = \beta.$

By substituting the above values for $a$, $b$, $\alpha$ and $\beta$, in the general equations, we obtain:

$$\text{Per cent fructose } (F) = \frac{1.571R - 1.032P}{2.836} = 0.554R - 0.364P \text{ (20° C.)}. \quad (7)$$

$$\text{Per cent arabinose } = \frac{R - 0.915F}{1.032} = 0.969R - 0.887F. \quad (8)$$

Correction for changes in temperature is made as in the previous cases.

The following analyses were made of two mixtures containing known amounts of fructose and arabinose.

| Taken. | | $R$ | $P$ | Temp. °C. | Found. | | Error. | |
|---|---|---|---|---|---|---|---|---|
| Fructose. | Arabinose. | | | | Fructose. | Arabinose. | Fructose. | Arabinose. |
| Per cent. | Per cent. | | | | Per cent. | Per cent. | Per cent. | Per cent. |
| 7.41 | 2.28 | 9.05 | −6.1 | 27° | 7.39 | 2.22 | −0.02 | −0.06 |
| 14.82 | 4.55 | 18.14 | −12.3 | 26° | 14.80 | 4.46 | −0.02 | −0.09 |
| | | | | | Average error..... | | −0.02 | −0.07 |

In the estimation of fructose and arabinose there is a wider range of specific rotations than with any other mixture of two sugars and a corresponding reduction in the experimental sources of error.

*Analysis of Mixtures of Xylose and Arabinose.* —

Reducing ratio of xylose to glucose = 0.983 = $a$.

Reducing ratio of arabinose to glucose = 1.032 = $b$.

$$\text{Polarizing ratio of xylose (10 per cent solution)} = \frac{+18.79}{+66.5} = 0.283 = \alpha.$$

$$\text{Polarizing ratio of arabinose} = \frac{+104.5}{+66.5} = 1.517 = \beta.$$

By substituting the above values for $a$, $b$, $\alpha$ and $\beta$, in the general equations, we obtain:

$$\text{Per cent xylose } (X) = \frac{1.571R - 1.032P}{1.252} = 1.255R - 0.824P. \quad (9)$$

$$\text{Per cent arabinose} = \frac{R - 0.983X}{1.032} = 0.969R - 0.953X. \quad (10)$$

The following analyses were made of four mixtures containing known amounts of xylose and arabinose.

| Taken. | | $R$ | $P$ | Temp. °C. | Found. | | Error. | |
|---|---|---|---|---|---|---|---|---|
| Xylose. | Arabinose. | | | | Xylose. | Arabinose. | Xylose. | Arabinose |
| Per cent. | Per cent. | | | | Per cent. | Per cent. | Per cent. | Per cent. |
| 1.98 | 6.14 | 8.35 | +10.2 | 25° | 2.05 | 6.13 | +0.07 | −0.01 |
| 3.96 | 12.28 | 16.66 | +20.3 | 25° | 4.17 | 12.17 | +0.21 | −0.11 |
| 6.05 | 1.73 | 7.85 | + 4.5 | 25° | 6.14 | 1.75 | +0.09 | +0.02 |
| 12.10 | 3.46 | 15.46 | + 8.8 | 25° | 12.14 | 3.42 | +0.04 | −0.04 |
| | | | | | Average error..... | | +0.10 | ±0.05 |

The five special cases, which have been selected, are sufficient to illustrate the principle and comparative accuracy of the combined polariscopic and reduction methods for analyzing mixtures of two reducing sugars. The method can also be used in analyzing mixtures which contain rhamnose, fucose, mannose, sorbose, etc.; the reducing factors of these less studied sugars have not as yet been definitely established. The method can also be applied to the analysis of mixtures containing the disaccharides, lactose and maltose, although, as previously stated, the reducing factors of the higher sugars do not have the same constancy as those of the monosaccharides. A reducing ratio to glucose of 0.7 for lactose hydrate and of 0.6 for maltose may be employed for Allihn's method with a fair degree of approximation.

The reducing factors of the different sugars for other methods, as those of Kjeldahl, Defren, Munson and Walker, etc., differ slightly from those found by Allihn's process. The chemist, so far as possible, should determine his own factors under the conditions of the method which he is using.

The degree of accuracy obtainable by a given combination of polariscopic and reduction methods is greatest, other conditions being equal, where there is the greatest difference between the specific rotations and reducing powers of the two sugars. The probable errors of the method are always indicated by the magnitude of the factors for $R$ and $P$ in the different equations. Thus an error in copper-reducing power is made six times as great in equation (3) as in equation (1), and an error in polarization five times as great in equation (3) as in equation 7. In mixtures of glucose and lactose, whose rotations are nearly alike, experimental errors are multiplied more than in the cases noted. Taking the polarizing ratio of lactose as 0.79 and the reducing ratio as 0.7, the percentage of lactose ($L$) is $L = 4.26\ P - 3.37\ R$.

## II. ANALYSIS OF MIXTURES CONTAINING THREE SUGARS

The indirect method of combining polarization and reducing power can also be applied, but with considerable limitations, to the analysis of mixtures containing three sugars.

**Methods Based upon a Determination of Total Sugars, Reducing Power, and Polarization.**—The calculation of three sugars in a mixture is sometimes made (1) from a determination of the total sugars, as by drying or by densimetric means, (2) from the reducing power and (3) from the polarization.

If three sugars $A$, $B$ and $C$ constitute a mixture, and no other substances are present, the percentages $x$, $y$ and $z$ of each may be expressed as follows:

$$x + y + z = T \text{ (total solids).} \quad (1)$$

$$ax + by + gz = R \text{ (reducing sugars as glucose).} \quad (2)$$

$$\alpha x + \beta y + \gamma z = P \text{ (polarization).} \quad (3)$$

Having determined $T$, $R$ and $P$, and knowing the reducing constants $a$, $b$, and $g$ and polarizing constants $\alpha$, $\beta$, and $\gamma$ of the three sugars, the percentages $x$, $y$ and $z$ of each may be calculated in certain cases with a fair degree of approximation. It frequently happens, however, in making calculations by this method that small experimental errors are enormously multiplied, so that the final results, even with mixtures of pure sugars, can be regarded as only very roughly approximate.

*Analysis of a Mixture Containing Glucose, Galactose and Fructose.*— As an example of the limitations above mentioned the problem of analyzing a mixture containing $x$ per cent glucose, $y$ per cent galactose and $z$ per cent fructose is taken. By substituting the reducing and polarizing constants previously employed for these three sugars in the general equations (1), (2) and (3) we obtain:

$$x + y + z = T,$$

$$x + 0.898\,y + 0.915\,z = R,$$

$$0.793\,x + 1.21\,y - 1.356\,z = P, \text{ at } 20^\circ \text{ C.},$$

whence,

$$z = \text{per cent fructose} = 1.957\,T - 1.638\,R - 0.401\,P, \text{ at } 20^\circ \text{ C.} \quad (1)$$

$$y = \text{per cent galactose} = 8.175\,T - 8.433\,R + 0.33\,P, \text{ at } 20^\circ \text{ C.} \quad (2)$$

$$x = \text{per cent glucose} = T - y - z. \quad (3)$$

It is seen that any experimental errors in determining total solids or reducing sugars are magnified in the calculation of galactose over eight times.

*Example.* — A solution containing 6.46 per cent glucose, 8.22 per cent galactose and 9.67 per cent fructose gave upon analysis the following results:

Total solids ($T$) by drying in vacuo 24.20 per cent; reducing sugars ($R$) as glucose 22.80 per cent; polarization ($P$ for 26 gms. in 100 c.c., 200-mm. tube at 20° C.) + 1.95° V. Substituting these values for $T$, $R$ and $P$ in the previous equations gives fructose 9.23 per cent; galactose, 6.21 per cent and glucose 8.76 per cent.

The relationships between experimental errors and the errors in calculated results in the above example are as follows:

| | Theoretical. | Found. | Error. | |
|---|---|---|---|---|
| Total solids | 24.35 | 24.20 | −0.15 | Experimental. |
| Reducing sugars as glucose | 22.70 | 22.80 | +0.10 | Experimental. |
| Polarization | +1.96 | +1.95 | −0.01 | Experimental. |
| Glucose | 6.46 | 8.76 | +2.30 | Calculated. |
| Galactose | 8.22 | 6.21 | −2.01 | Calculated. |
| Fructose | 9.67 | 9.23 | −0.44 | Calculated. |

It is seen that a combination of very slight experimental errors introduces an error of over 2 per cent in the calculation of glucose and galactose.

*Analysis of a Mixture Containing Glucose, Fructose and Sucrose.* — When one of the three sugars in a mixture is non-reducing, the calculation by the above indirect method can frequently be made with a much greater degree of accuracy. Thus for a mixture containing $x$ per cent glucose, $y$ per cent fructose and $z$ per cent sucrose, the three general equations would give:

$$x + y + z = T,$$
$$x + 0.915\,y = R,$$
$$0.793\,x - 1.356\,y + z = P, \text{ at } 20^\circ \text{ C.},$$

whence,

$$y = \text{per cent fructose} = 0.461\,(T - P) - 0.096\,R, \text{ at } 20^\circ \text{ C.} \quad (4)$$
$$x = \text{per cent glucose} = R - 0.915\,y. \quad (5)$$
$$z = \text{per cent sucrose} = T - x - y. \quad (6)$$

It is seen that in a mixture of glucose, fructose and sucrose there is a division, rather than a multiplication, of experimental errors in the calculation.

*Example.* — A solution containing 5.43 per cent fructose, 10.02 per cent glucose and 16.16 per cent sucrose gave upon analysis the following results: Total solids ($T$) by drying in vacuo 31.50 per cent; reducing sugars ($R$) as glucose by Allihn's method, 15.24 per cent; polarization ($P$, 26 gms. in 100 c.c., 200-mm. tube at 25° C.) + 17.05° V. Substituting these values for $T$, $R$ and $P$ in equations (4), (5) and (6) gives 5.40 per cent fructose, 10.30 per cent glucose and 15.80 per cent sucrose.

The relationship between experimental errors and the errors in calculated results in the above example are as follows:

| | Theoretical. | Found. | Error. | |
|---|---|---|---|---|
| Total solids | 31.61 | 31.50 | −0.11 | Experimental. |
| Reducing sugar as glucose | 14.99 | 15.24 | +0.25 | Experimental. |
| Polarization | +16.75 (20° C.) | +17.05 (25° C.) | +0.30 | Experimental. |
| Fructose | 5.43 | 5.20 | −0.23 | Calculated. |
| Glucose | 10.02 | 10.48 | +0.46 | Calculated. |
| Sucrose | 16.16 | 15.82 | −0.34 | Calculated. |

It is seen that the calculation by this method gives a very good approximation, notwithstanding the influence of rather large experimental errors (due to polarizing at 25° C. instead of 20° C. and to the slight reducing action of sucrose).

*Analysis of a Mixture Containing Glucose, Maltose and Dextrin.* — Several indirect methods, based upon determinations of total solids, reducing power and polarization have been proposed for the analysis of starch-conversion products which contain the three carbohydrates, glucose, maltose and dextrin.

In the method proposed by Allen* the $[\alpha]_D$ of glucose is taken as +52.7, of maltose as +139.2 and of dextrin as +198.0. The copper-reducing power of glucose is taken as 1, of maltose as 0.62, and of dextrin as 0. The sum of the glucose ($g$), maltose ($m$) and dextrin ($d$) is taken as the total organic solids (O), and is found by subtracting the percentage of ash from the percentage of total dry substance. The three general equations used by Allen are:

$g + m + d = \mathrm{O}$ (organic solids).

$g + 0.62\,m = K$ (copper-reducing power by O'Sullivan's method).

$52.7\,g + 139.2\,m + 198\,d = 100\,S$ (specific rotation).

By substituting the first equation in the last and transposing we obtain:

$$139.2\,m = 100\,S - 52.7\,g - 198\,(\mathrm{O} - g - m);$$

by substituting $K - 0.62\,m$ for $g$ in the preceding equation and transposing, we obtain:

$$31.3\,m = 100\,S - 52.7\,K - 198\,(\mathrm{O} - K);$$

dividing the above by 100 we obtain:

$$m = \left(S - \frac{52.7\,K + 198\,(\mathrm{O} - K)}{100}\right) \div 0.313. \qquad (1)$$

$$g = K - 0.62\,m. \qquad (2)$$

$$d = \mathrm{O} - g - m. \qquad (3)$$

* Allen's "Commercial Organic Analysis" (1901), Vol. I, 365.

Equation (1) of Allen, expressed in its simplest decimal form, becomes

$$m = 3.195\,S + 4.642\,K - 6.326\,O. \qquad (4)$$

If the sample be polarized upon a saccharimeter, where the ratio of the scale reading for the normal weight to specific rotation will be as the $[\alpha]_D$ of sucrose (+66.5) is to 100, the factor for the saccharimeter reading of a normal weight would be for equation (4)

$$100 : 66.5 :: 3.195 : x = 2.125.$$

Equation (1) of Allen modified for the polarization ($P$) of a sucrose normal weight upon a saccharimeter would then be:

$$m = 2.125\,P + 4.642\,K - 6.326\,O.$$

In the analysis of starch-conversion products the total solids are frequently calculated by means of the solution factor 3.86. When this is done, a correction must be introduced for the variations from 3.86 in the solution factors of the different ingredients. The solution factor of the mineral matter, or ash, in a conversion product has been placed at 8;* taking as the solution factors† of glucose ($g$), maltose ($m$) and dextrin ($d$), the values 3.83, 3.92 and 4.21 respectively (p. 31), then the equation for total solids ($T$) as calculated from the specific gravity by the solution factor 3.86 (usually written $T_{3.86}$) would be:

$$\frac{3.86}{3.83}g + \frac{3.86}{3.92}m + \frac{3.86}{4.21}d + \frac{3.86}{8}a = T_{3.86}.$$

Knowing the percentage of ash ($a$), the equation of Allen for organic solids would be:

$$\frac{3.86}{3.83}g + \frac{3.86}{3.92}m + \frac{3.86}{4.21}d = T_{3.86} - \frac{3.86}{8}a.$$

If the reducing power be expressed in percentage of the solids as calculated by the factor 3.86 (written $K_{3.86}$) then

$$\frac{3.86}{3.83}g + \frac{3.86}{3.92}0.62\,m = K_{3.86}.$$

In the same way if the $[\alpha]_D$ of the solids, as calculated by the factor

* Allen's "Commercial Organic Analysis" (1901), Vol. I, 376.

† It is noted that the solution factors of glucose, maltose and dextrin increase in the order of their specific rotations. From this relationship Rolfe (J. Am. Chem. Soc., **19,** 698) has derived a general equation $\Sigma = 0.004023 - 0.000001329\,(195 - [\alpha]_D)$, for calculating the specific gravity influence of any acid-hydrolyzed starch solution, when the value for $[\alpha]_D$ (obtained by the factor 0.00386 between the densities 1.035 and 1.045) is known. The value for $\Sigma$ multiplied by 1000 will give of course the O'Sullivan solution factor.

3.86, be used instead of the $[\alpha]_D$ of the moist product, then, using the values of Allen for the $[\alpha]_D$ of glucose, maltose and dextrin

$$\frac{3.86}{3.83}52.7\,g + \frac{3.86}{3.92}139.2\,m + \frac{3.86}{4.21}198\,d = 100[\alpha]_{D_{3.86}}.$$

Several other methods of calculating maltose, glucose and dextrin have been proposed. These are similar to that of Allen, except that slightly different values are used for the polarizing and reducing constants.

It is seen that in the calculation of maltose by Allen's method any experimental errors in determining organic solids, reducing power or specific rotation are greatly multiplied. The value of the method in the analysis of hydrolyzed starch products is still further diminished by the fact that no account is taken of isomaltose and of the various reversion products which are always present in materials of high conversion. Any reducing power and rotation due to other substances than glucose, maltose and dextrin affect the accuracy of the method to a marked degree. Furthermore the dextrins of starch conversion are of a mixed character with different rotations and reducing powers, so that the selection of an initial dextrin of $[\alpha]_D + 198$ and negative reducing power is largely arbitrary. The percentages of glucose, maltose and dextrin in starch-conversion products, as calculated from determinations of organic solids, reducing power and polarization are, therefore, largely conventional quantities; the latter, when properly understood, may serve, however, as a valuable means of comparison.

The methods of estimating three sugars in mixture which depend upon a determination of total sugars become largely valueless in the case of such products as molasses, fruit juices, honeys, etc., which contain varying amounts of organic and mineral salts, gums, and acids. With such materials a determination of dry substance, or of organic solids, gives too high a percentage of total sugars, and the results of the calculation may even lack the value of an approximation. It is, therefore, always the best plan to determine as many of the sugars as possible in a mixture by direct means.

**Methods of Calculating the Percentages of Three Sugars from the Combined Reducing Power and Polarization and the Direct Determination of One Sugar.** — If in a mixture of three sugars containing $x$ per cent $A$, $y$ per cent $B$ and $z$ per cent $C$, the percentage $z$ of $C$ be determined by direct means, then $x$ and $y$ can be calculated by means of the following equations:

$$ax + by + gz = R \text{ (total reducing sugars as glucose)},$$
$$\alpha x + \beta y + \gamma z = P \text{ (polarization)},$$
$$z = Z \text{ (direct determination)},$$

whence
$$ax + by = R - gZ,$$
$$\alpha x + \beta y = P - \gamma Z.$$

Having determined $R$, $P$ and $Z$ and knowing the reducing and polarizing constants of the three sugars, the percentages $x$ and $y$ can be calculated as described on page 477, for mixtures of two sugars.

Several applications of the method will be described.

*Analysis of a Mixture Containing Glucose, Fructose and Sucrose.* — The sucrose is best determined by the methods of inversion, using either the process of double polarization or that of copper reduction. If the polariscopic method be used, the inversion is best accomplished by means of invertase in order to eliminate the influence of the acid upon the rotation of fructose.

Knowing the percentage ($S$) of sucrose in a mixture containing $x$ per cent glucose and $y$ per cent fructose, and no other optically active or reducing substances, the percentages $x$ and $y$ can be calculated by means of the two equations:

$$x + 0.915\,y = R \text{ (reducing sugars as glucose)},$$
$$0.793\,x - 1.356\,y + S = P, 20^\circ \text{ C.} \left(\begin{matrix}\text{polarization of a sucrose normal}\\ \text{weight on a saccharimeter}\end{matrix}\right)$$

whence, $y$ = per cent fructose $= \dfrac{0.793\,R + S - P}{2.08}$, at 20° C. (1)

$$x = \text{per cent glucose} = R - 0.915\,y. \quad (2)$$

The determination of $R$ will be a little too high, unless a correction is made for the slight reducing action of sucrose upon Fehling's solution. This correction can be made by using an empirical formula, such as proposed by Browne for Allihn's method (p. 427), or by using the special methods and tables for determining reducing sugars in presence of sucrose.

*Example.* — The solution employed in the previous example (p. 485) gave by the method of inversion 16.27 per cent of sucrose ($S$); substituting this and the previous values, $R = 15.24$, and $P = +17.05$ at 25° C., in equation (1) we obtain:

$$\text{Fructose} = \frac{0.793\,(15.24) + 16.27 - 17.05}{2.08} = 5.43 \text{ per cent.}$$

$$\text{Glucose} = 15.24 - 0.915(5.43) = 10.27 \text{ per cent.}$$

These percentages agree more closely than in the previous example with the actual amounts of sugars taken, viz.: 5.43 per cent fructose, 10.02 per cent glucose and 16.16 per cent sucrose.

*Analysis of a Mixture Containing Glucose, Maltose and Dextrin.* — In addition to the method of Allen previously described, several processes have been devised for determining glucose, maltose and dextrin in starch-conversion products, which are based upon a direct determination of the dextrin.

*Determination of Dextrin.* — Several methods have been proposed for the direct estimation of dextrin in presence of other carbohydrates, but none of these has been found to give perfectly reliable results.

The dextrin is sometimes precipitated from the sirupy solution by adding a large excess of hot 95 per cent alcohol, and stirring, after which the precipitate of dextrin is allowed to subside. The clear solution when deposition is complete is decanted through a filter, the dextrin dissolved in a little water and again precipitated by adding alcohol as before. The process is repeated for a third time, after which the precipitate is washed into a platinum evaporating dish, and dried and weighed. The residue is then ignited and the weight of ash deducted from the weight of dried alcohol precipitate; the difference is estimated as dextrin. The difficulty with this method of estimation is to precipitate all of the dextrin without occluding any of the glucose or maltose. The dextrin after repeated precipitations with alcohol still reduces Fehling's solution; this may be due, however, to the presence of reducing maltodextrins as well as to the occlusion of sugars.

Methods based upon a destruction of reducing sugars by fermentation or oxidation, and then calculating the residual polarizing power to dextrin have already been referred to (p. 301). The principal objection to the fermentation method is that most yeasts ferment or modify dextrin to a greater or less degree so that the residual polarizing power does not represent that of the dextrins originally present. In Wiley's method (p. 306) of destroying reducing sugars by oxidation with alkaline mercuric cyanide, it has been found that the polarizing power of maltose is not completely destroyed and that the dextrins themselves undergo partial oxidation to dextrinic acid.

Owing to the limitations of the methods just described it is evident that the percentages of dextrin thus determined have only a nominal value.

Assuming that the residual polarizing power ($P'$), after destroying maltose and glucose, is due to an unchanged dextrin of $[\alpha]_D + 193$, and calling the $[\alpha]_D$ of glucose $(g) + 53$ and of maltose $(m) + 138$, and supposing the relative reducing powers of glucose and maltose to be 100

and 62* respectively, the calculation of the percentages $g$, $m$ and $d$ in a starch-conversion product is made by Wiley† as follows:

$$g + 0.62\,m = R \text{ (total reducing sugars as glucose).} \quad (1)$$

$$53\,g + 138\,m + 193\,d = 100\,P, \ (P = [\alpha]_D \text{ of product}). \quad (2)$$

$$193\,d = 100\,P', \ (P' = [\alpha]_D \text{ after destroying } g \text{ and } m). \quad (3)$$

Subtracting (3) from (2) gives

$$53\,g + 138\,m = 100\,(P - P'). \quad (4)$$

Multiplying (1) by 53 and subtracting from (4) gives

$$105.14\,m = 100\,(P - P') - 53\,R, \quad (5)$$

whence

$$m = \frac{100\,(P - P') - 53\,R}{105.14} = 0.951\,(P - P') - 0.504\,R. \quad (6)$$

$$g = R - 0.62\,m. \quad (7)$$

$$d = \frac{100\,P'}{193}. \quad (8)$$

*Example.* — A sample of midzu ame (Japanese glucose) was analyzed by Wiley with the following results:

$[\alpha]_D$ before fermentation $= +\,132.6 = P$.

$[\alpha]_D$ after fermentation $= +\,59.2 = P'$.

Total reducing sugars as glucose $= 33.33$ per cent $= R$.

Substituting these values in equations (6), (7) and (8) gives:

Maltose $= 0.951(132.6 - 59.2) - 0.504(33.33) = 53.01$ per cent,

Glucose $= 33.33 - 0.62(53.01) = 0.47$ per cent,

Dextrin $= \frac{100\,(59.2)}{193} = 30.67$ per cent.

If the sample be polarized upon a saccharimeter the factor for the scale readings, $P$ and $P'$ of a sucrose normal weight would be for equation (6)

$$100 : 66.5 :: 0.951 : x = 0.632.$$

Equation (6) of Wiley modified for the polarizations of a sucrose normal weight upon a saccharimeter would then be

$$m = 0.632\,(P - P') - 0.504\,R.$$

Equation (8) of Wiley modified for calculating dextrin from the saccharimeter reading ($P'$) of a sucrose normal weight would be

$$\frac{193}{66.5}\,d = P', \text{ whence } d = \frac{P'}{2.902}.$$

The criticisms on page 488 of the indirect method of estimating glucose, maltose and dextrin from organic solids, polarization and reducing power apply also to the method of calculation just described.

* The ratio 62, or in decimal form 0.62, is strictly true only for O'Sullivan's method. The factor is less than this for other processes of copper reduction.

† Wiley's "Agricultural Analysis" (1897), Vol. III, 288.

Owing to the mixed character of the dextrins in starch conversion products, the selection of a dextrin of $[\alpha]_D = +193$, or of any other fixed value, as a basis of calculation is largely conventional. The presence of the unfermentable reducing sugar isomaltose and of optically-active reversion products also affects the accuracy of the method. Owing to these reasons, as well as to the general unreliability of the methods for estimating dextrin, the results of such calculations have frequently no absolute scientific value.

**Applications of the Method to Other Sugar Mixtures.** — The general principle of combining the results of polariscopic and reduction methods with those of a direct determination in analyzing mixtures of three sugars has been sufficiently indicated, and additional examples need not be given. Such schemes of analysis obviously admit of unlimited extension. If one of the three sugars is a pentose or methylpentose, its percentage may be determined from the yield of furfural- or methylfurfural-phloroglucide; mannose may be determined from the yield of phenylhydrazone; lactose or galactose from the yield of mucic acid; raffinose by the method of inversion; etc. In combining the results of such direct determinations with those of polarization and reducing power, the chemist must consider in each case the limitations of the methods used and the extent to which experimental errors are multiplied in the calculation.

The final test of accuracy consists in applying the method to the analysis of mixtures containing known amounts of the several sugars, and this verification should be made whenever possible.

### III. ANALYSIS OF MIXTURES CONTAINING FOUR SUGARS

Schemes of analysis have also been proposed for the analysis of mixtures containing four sugars, in which case, however, two of the members present must usually be determined by direct means.

As a single illustration of such methods the following scheme is given for analyzing a mixture containing $g$ per cent glucose, $f$ per cent fructose, $s$ per cent sucrose and $x$ per cent xylose.

$$0.793\,g - 1.356\,f + s + 0.283\,x = P \left( \begin{matrix} \text{polarization of a sucrose normal} \\ \text{weight upon a saccharimeter} \end{matrix} \right). \quad (1)$$

$$g + 0.915\,f + 0.983\,x = R \text{ (total reducing sugars as glucose).} \quad (2)$$

$$s = S \text{ (sucrose determined by method of inversion).} \quad (3)$$

$$x = X \text{ (xylose determined from yield of furfural phloroglucide).} \quad (4)$$

Substituting the known values of $S$ and $X$ in (1) and (2) gives:

$$0.793\,g - 1.356\,f = P - S - 0.283\,X. \quad (5)$$

$$g + 0.915\,f = R - 0.983\,X \quad (6)$$

Multiplying (6) by 0.793 and combining with (5) gives:

$$f = \text{per cent fructose} = \frac{S + 0.793\,R - P - 0.497\,X}{2.082}.$$

$$g = \text{per cent glucose} = R - 0.915\,f - 0.983\,X.$$

The application of such formulæ as the above to the analysis of complicated mixtures of sugars usually involves, however, such a combination and multiplication of experimental errors, that a scheme of calculation, perfectly correct in theory, is shown in practice to be almost valueless.

It is scarcely necessary to remark that in working with unknown mixtures of sugars, each of the constituents present must be identified by careful qualitative tests before beginning the analysis.

For a description of other methods and schemes which have been proposed for analyzing different mixtures of sugars, the chemist is referred to Lipmann.*

* "Chemie der Zuckerarten," Vol. I, 616–623; 894–899.

See also Wiechmann's "Sugar Analysis" (1898), and the papers by Halenke and Möslinger (Z. analyt. Chem., **34,** 263) and by Geelmuyden (Z. analyt. Chem., **48,** 137) for other examples of calculation.

# CHAPTER XVII

## MISCELLANEOUS APPLICATIONS

The present chapter will give several practical applications of the principles and methods previously described to a few selected problems of technical sugar analysis. A large number of such applications have already been considered and a description of these will be passed over. The methods will be grouped under three main divisions of products: (1) Sugar-factory products; (2) Starch-conversion products; (3) Food products.

### Sugar-factory Products

In addition to the analytical methods, previously considered, a few definitions of common terms and several descriptions of illustrative commercial methods will be given. For the application of methods to sugar-factory control, the technical works of Geerligs, Mittelstaedt, Morse, Spencer, Pellet and Metillon and others should be consulted.

**Coefficient of Purity.** — The coefficient of purity of a juice, sirup, molasses, sugar, etc., is the percentage of sucrose in the total solid matter of the product. The term, which is also called "quotient of purity," "degree of purity," "purity" or "exponent," has been variously interpreted, and the chemist must distinguish carefully between the true and the apparent coefficient of purity.

The *true coefficient of purity* is the percentage of actual sucrose in the total solid matter as determined by the method of drying.

The *apparent coefficient of purity* is usually taken as the ratio of the direct polarization to 100 parts of apparent solids as calculated from the degrees Brix, or by other indirect means.

*Example.* — A sugar-cane molasses gave upon analysis the following result :

| | |
|---|---|
| Total solids by actual drying | 75.10 per cent |
| Total solids by degrees Brix | 77.10 per cent |
| Total solids by refractometer | 74.20 per cent |
| Direct polarization | 42.20 |
| Sucrose by method of inversion | 45.70 per cent |

$$\text{True coefficient of purity} = \frac{45.70}{75.10} \times 100 = 60.85.$$

$$\text{Apparent coefficient of purity} = \frac{42.20}{77.10} \times 100 = 54.73. \qquad (1)$$

$$\text{Apparent coefficient of purity} = \frac{42.20}{74.20} \times 100 = 56.87. \quad (2)$$

Sometimes the true percentage of sucrose is used in calculating apparent purity in which case

$$\text{Apparent coefficient of purity} = \frac{45.70}{77.10} = 59.27. \quad (3)$$

$$\text{Apparent coefficient of purity} = \frac{45.70}{74.20} = 61.59. \quad (4)$$

The coefficient of purity of sugar-cane or sugar-beet juices is often loosely applied to the entire cane or beet.

Numerous tables and formulæ have been calculated for converting apparent into true purities, but these can only be used upon the special classes of products for which they were designed.

**Determination of Ash.** — The determination of ash is of great importance in the technical analysis of sugar products. Several methods of the Association of Official Agricultural Chemists * are given.

*Direct Incineration.* — Heat from 5 to 10 gms. of sugar, molasses, etc., in a platinum dish of from 50- to 100-c.c. capacity at 100° C. until the water is expelled, and then slowly over a flame until intumescence ceases. Then place the dish in a muffle and heat at low redness until a white ash is obtained.

*Soluble and Insoluble Ash.* — Add water to the ash in the platinum dish after weighing the total ash in the previous method, heat nearly to boiling, filter through ash-free filter paper and wash with hot water until the filtrate and washings amount to about 60 c.c. Return the filter paper and contents to the platinum dish, carefully ignite and weigh. The residue is the weight of insoluble ash. The difference between insoluble and total ash gives the soluble ash.

Owing to the difficulty of obtaining a perfectly carbon-free ash and to the danger of expelling volatile salts during ignition Scheibler† has recommended burning the sample in presence of sulphuric acid.

*Ignition with Sulphuric Acid.* — Saturate the sample with sulphuric acid, dry, ignite gently, then burn in a muffle at low redness. Deduct one-tenth of the weight of the ash, then calculate the per cent.

Instead of deducting one-tenth, to correct for the weight of combined sulphuric acid, Girard and Violette propose the deduction of one-fifth.

*Preparation of Ash for Quantitative Examination.* — When it is desired to obtain a pure carbon-free ash for quantitative examination the following method should be used: Carbonize the mass at a low heat,

* Bull. 107 (revised), U. S. Bur. of Chem., pp. 67 and 68.

† Stammer's Jahresbericht, **4**, 221; **7**, 267.

dissolve the soluble salts with hot water, burn the residual mass to whiteness, add the solution of soluble salts; and evaporate to dryness at 100 °C., ignite gently, cool in a desiccator and weigh.

**Determination of Organic Matter.** — The percentage of ash deducted from the percentage of total solids gives the percentage of organic matter.

**Determination of Non-sugar.** — The percentage of sucrose deducted from the percentage of total solids gives the percentage of non-sugars.

**Determination of Organic Non-sugar.** — The percentage of ash deducted from the percentage of non-sugar gives the organic non-sugar.

**Saline Quotient.** — This coefficient is found by dividing the percentage of sucrose by the percentage of ash.

**Glucose Ratio.** — The glucose ratio, or coefficient, represents the parts of glucose per 100 of sucrose. It is found by multiplying the percentage of reducing sugars by 100 and dividing by the percentage of sucrose.

The determination of the glucose ratio is of great importance in sugar-house control. Any increase in this coefficient during clarification or evaporation indicates a partial inversion of sucrose.

**Determination of Extraction.** — The term extraction has been given several meanings in consequence of which occasional confusions and misunderstandings have arisen.

In Louisiana and Cuba, extraction indicates the percentage of undiluted juice which is obtained from a given weight of cane. Thus, if 2000 lbs. of cane give 1500 lbs. of undiluted juice the extraction is $\frac{1500}{2000} \times 100 = 75$ per cent. If the juice has been diluted owing to saturation (i.e., spraying the ground cane with water before regrinding), its equivalent in undiluted juice must first be determined before making the calculation.

In the Hawaiian Islands, extraction means the percentage of sucrose in the cane that is obtained in the mixed juices and is calculated by the formula $\frac{\text{per cent sucrose in mixed juice} \times \text{weight of mixed juice}}{\text{per cent sucrose in cane} \times \text{weight of cane}} \times 100.$

*Example.* — 2000 lbs. of cane containing 15 per cent sucrose gave 2300 lbs. of mixed diluted juice which polarized 12.4. Then

$$\frac{12.4 \times 2300}{15 \times 2000} \times 100 = 95.07 \text{ per cent extraction.}$$

**Determination of Acidity and Alkalinity of Sugar Products.** *Herzfeld's Method.* — The determination of the acidity and alkalinity of sugar products is at times a matter of considerable importance. The Herzfeld or German official method for determining the

acidity and alkalinity of raw sugars is selected for description. The following solutions are used:

(1) *Phenolphthalein.* — One part of phenolphthalein is dissolved in 30 parts of neutral 90 per cent alcohol.

(2) *Neutral Water.* — Ten liters of freshly boiled distilled water are treated with 5 c.c. of the phenolphthalein solution and sufficient dilute alkali (see under 4) added to produce a permanent pink tinge. The water should be prepared several hours before use, but should not be used after one or two days as the indicator loses its sensibility.

(3) *Standard Sulphuric Acid.* — A $n/280$ sulphuric acid solution is prepared, 1 c.c. of which is equivalent to 0.0001 gm. CaO.

(4) *Standard Sodium Hydroxide.* — A $n/280$ sodium-hydroxide solution is prepared, 1 c.c. of which exactly neutralizes 1 c.c. of the standard acid.

Ten grams of the sugar are dissolved in 100 c.c. of the neutral water * in a porcelain evaporating dish. If the pink tinge of the neutral water is discharged the sugar is acid and the acidity is measured by noting the volume of standard alkali necessary to restore the original color. If the pink tinge of the neutral water is reddened the sugar is alkaline and the alkalinity is measured by noting the volume of standard acid necessary to bring back the original tint. If the end-point of the titration is over-run, the solution is titrated back with acid or alkali as the case may be. The acidity or alkalinity of the sugar is then expressed in the equivalent percentage of CaO. Thus 10 gms. of a sugar requiring 30 c.c. of standard acid for neutralization would have an alkalinity of 0.03 per cent CaO.

**Normal Juice.** — The true normal juice is the mixed juice as it actually exists in the tissues of the cane or beet. It is impossible to obtain this true normal juice by any method of pressing or milling for reasons explained on page 232, so that its composition and percentage must be calculated by indirect means. In cane-sugar factories it is often customary to call the undiluted juice of the first mill the normal juice and to make all calculations upon this basis. A more correct practice is to determine the degrees Brix and polarizations of the different mill juices and then by means of empirical factors, established for the conditions of each factory, to calculate the approximate percentage and composition of the normal juice.

* With dark sugars a larger volume of the neutral water must be taken. Cross (Int. Sugar J. **13**, 305), in a modification of Herzfeld's method, employs 200 c.c. of neutral water.

**Dutch Standard.** — The Dutch standard consists of a series of samples of cane sugar ranging in color from a very dark No. 7 to an almost white No. 25. These samples are put up each year in sealed bottles by two firms in Holland, under the direction of the Netherlands Trading Society, and are sent to different parts of the world as color standards for classifying sugars in the assessment of duty. The relation between color and composition is such a loose one that the Dutch standard has purely an arbitrary value.

**Calculation of Rendement.*** — The rendement is the yield of pure crystallized sucrose which can be obtained from a raw product. The various formulæ, employed in its calculation, subtract from the polarization, or sucrose content, of the product a certain quantity which is taken to represent the melassigenic influence of the ash or other non-sugars. One of the most common methods of calculation is that first proposed by Monnier in France in 1863; Monnier assumed that 1 part of mineral impurities prevented the crystallization of 5 parts of sucrose, and so calculated the yield of crystallizable sugar by subtracting 5 times the percentage of ash from the polarization of the raw product. This method of calculation is very largely used in the valuation of raw beet sugars. For cane sugars the following formula is often used: Rendement = Polarization $-(5 \times$ per cent ash $+$ per cent invert sugar).

Monnier's formula for calculating rendement is used, however, more in other countries than in France itself. The method most used in France at present is to subtract from the polarization 4 times the percentage of ash and twice the percentage of invert sugar; from this remainder 1.5 per cent additional is then deducted as the loss in refining. In 1893 the German Refiners' Association introduced a method for calculating rendement which consisted in multiplying the percentage of total non-sugars by $2\frac{1}{4}$ and subtracting the product from the polarization. This "non-sugar yield" was found, however, to be less satisfactory than the "ash yield" and a return was made to the old method of Monnier. The number of methods used by different associations and factories for calculating rendement is almost unlimited.

**Determination of Crystal Content.** — The calculation of rendement by formula is unsatisfactory for the reason that the variations in melassigenic influence of the non-sugars are not considered. A direct determination of the sucrose crystals in a raw sugar has, therefore, been proposed as a better means of determining the refining yield.

* For a very full discussion of methods for calculating the refining value, "net analysis," or rendement of raw sugars see Mittelstaedt's "Technical Calculations for Sugar Works."

*Method of Payen.* — The different methods for determining sugar content are all modifications of the early process of Payen,* which consisted in washing the adhering sirup from the crystals of raw sugar by means of 88 per cent alcohol, saturated with sugar and containing 50 c.c. of strong acetic acid per liter. The object of the acid was to break up saccharates and promote the solution of calcium carbonate and other mineral matter. The method of Payen was displaced in 1871 by the following modification of Scheibler.†

*Scheibler's Method for Determining Crystal Content.* — The four washing liquids used in Scheibler's method have the following composition:

(1) 85 per cent alcohol containing 50 c.c. of strong acetic acid per liter is saturated by shaking with an excess of powdered sucrose.

(2) 92 per cent alcohol saturated with sucrose as (1).

(3) 96 per cent alcohol saturated with sucrose as (1).

(4) A mixture containing 2 volumes of absolute alcohol and 1 volume of ether.

Stock solutions (1), (2) and (3) are preserved in large double-neck bottles (Fig. 184), which are filled, as is also the siphon tube *S*, with lumps of loaf sugar. The tube *T* contains calcium chloride for preventing absorption of moisture from the air. The solutions should not be exposed to wide changes in temperature.

In making the determination a half-normal weight of the ground sample of sugar is placed in a 50-c.c. graduated flask *F*, which is closed with a two-hole stopper. One hole of the latter is fitted with the inlet tube *I*, through which the washing liquids are added, and the other with the outlet tube *O*, through which they are withdrawn. The tube *O* extends to the bottom of the flask and at its lower enlarged end is fitted with a filtering plug of felt. The large bottle *B*, which receives the spent washing liquids, is connected by the opening of its stopper to the outlet tube *O* of the sugar flask and by the side opening to a suction pump.

The alcohol-ether solution (4) is first run into the flask *F*, using about 2 volumes to 1 volume of sugar. After standing 10 minutes, with occasional shaking, the liquid is sucked off into *B*. The alcohol-ether removes moisture from the sugar and at the same time precipitates sucrose from the film of sirup adhering to the crystals. The sugar is then treated in exactly the same way with solutions (3) and (2); the latter remove the traces of alcohol and ether left from (4) and prepare the sugar for the action of solution (1) which accomplishes the

* Dingler's Polytech. Journal (1846), **100**, 127.

† Full descriptions of Scheibler's experiments are given in Stammer's Jahresbericht, Vols. **12** and **13** (1872 and 1873).

chief part of the washing. Solution (1) is next added, using the same proportions as before. After shaking 10 to 15 minutes the spent liquor is withdrawn, and a second portion of (1) added; the process is continued with (1) until the washings become colorless. The sugar is

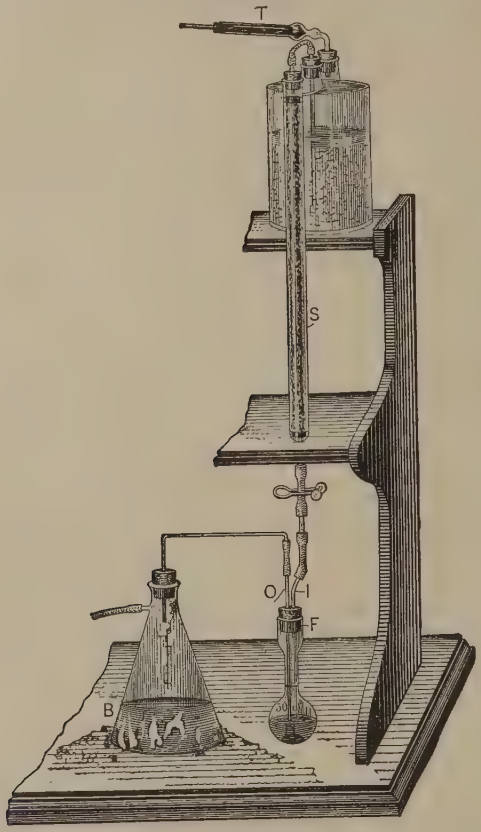



Fig. 184. — Scheibler's apparatus for determining crystal content of raw sugars.

then treated with solutions (2), (3) and (4) in the order named. After removing as much of (4) as possible, the flask *F* is gently warmed, while a strong current of air is drawn through to remove the last traces of alcohol and ether. The connections are then removed from *F*, any particles of sugar adhering to the tube *O*, or plug of felt, washed into the flask, and sufficient water added to dissolve the contents. A few

drops of lead reagent are added, and the volume completed to 50 c.c. The solution is then filtered and polarized; the saccharimeter reading gives the percentage of sucrose crystals in the sugar.

The method of Scheibler for determining crystal content has not given satisfactory results and is at present but little used. It has been found that a considerable precipitation of sucrose may take place from adhering wash liquors especially upon contact with the alcohol-ether. The precipitation of sucrose from the molasses in the sugar is also objectionable, especially when it is desired to calculate the composition and amount of such molasses.

*Koydl's Method for Determining Crystal Content.* — In order to reduce the above-named errors and simplify the manipulation, Koydl* has recently modified the Payen-Scheibler method as follows:

The following five washing liquids are used:

(1) 82 per cent (by weight) alcohol containing 50 c.c. concentrated acetic acid per liter.

(2) 86 per cent (by weight) alcohol containing 25 c.c. concentrated acetic acid per liter.

(3) 91 per cent (by weight) alcohol.

(4) 96 per cent (by weight) alcohol.

All of the above solutions are saturated with sucrose in the cold, and kept over lump sugar in stock bottles.

(5) Common absolute alcohol.

In making the determination 50 gms. of sugar are weighed into a beaker of ordinary form, 18 cm. high; 250 c.c. of solution (1) are measured into a wash bottle from which a sufficient quantity is added to the beaker until the sugar is covered about 1 cm. deep. After well mixing, the solution is poured through a weighed filter paper (16 cm. diameter) in a covered funnel. The process is repeated several times, the sugar being finally transferred to the filter and washed with solution (1) until the 250 c.c. are used. When the filter has drained completely, 50 c.c. of solutions (2), (3) and (4) are poured in successive portions upon the sugar, each liquid being allowed to filter off before adding the one following. The sugar is then washed with 100 c.c. of (5) taking care to wash well the edges of the paper. When the alcohol has filtered completely, the paper and its contents are dried in an oven and then weighed. The weight of product multiplied by two gives the crystal content of the sugar.

Koydl's method has been found to give results which are approximately quantitative, when the requirements of uniform temperature,

* Oester. Ungar. Z. Zuckerind., (1906), 277.

saturation of solutions and other details are carefully maintained. With variations from these requirements a considerable error may result from solution or precipitation of sucrose. A certain amount of gum and mineral matter is always precipitated from the adhering molasses by the alcoholic solutions; the final crystals when dried polarize from 99.4 to 99.8 and contain about 0.2 per cent organic non-sugar and 0.15 per cent ash.

Results of analyses of several beet sugars giving the composition, rendement (polarization less 5 times ash) and crystal content by Koydl's method are given in Table LXXXIV which is taken from results by Ehrlich.*

TABLE LXXXIV

| No. | Polarization. | Moisture. | Ash. | Organic non-sugar. | Rendement. | Crystals (Koydl's method). | | Molasses (100 less per cent crystals). |
|---|---|---|---|---|---|---|---|---|
| | | | | | | I. | II. | |
| | | Per cent. | Per cent | Per cent. | | Per cent. | Per cent. | Per cent. |
| 1 | 96.30 | 1.49 | 0.81 | 1.40 | 92.25 | 93.32 | 93.14 | 6.77 |
| 2 | 95.85 | 1.24 | 1.17 | 1.74 | 90.00 | 92.04 | 92.28 | 7.84 |
| 3 | 95.10 | 2.04 | 1.13 | 1.73 | 89.45 | 90.83 | 90.87 | 9.15 |
| 4 | 94.50 | 1.63 | 1.41 | 2.46 | 87.45 | 91.20 | 91.17 | 8.82 |
| 5 | 94.40 | 1.84 | 1.37 | 2.39 | 87.55 | 89.87 | 90.10 | 10.02 |

It is seen that no strict proportionality exists between polarization, rendement and crystal content. Sugars 4 and 5 have practically the

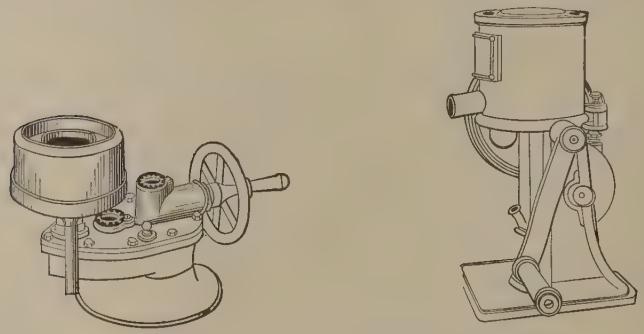


Fig. 185. — Laboratory hand centrifugals.

same polarization and rendement, but sugar 4 contains over 1 per cent more crystals and over 1 per cent less molasses than sugar 5, and is, therefore, more valuable for refining purposes.

* Z. Ver. Deut. Zuckerind., **59**, 548, 995.

There are other modifications of Payen's method for determining crystal content, but none equal in practicability to those of Scheibler and Koydl.* Such methods have found their chief value not in the work of routine but in providing a control upon other processes.

In many European refineries the crystal content of raw sugars, massecuites, etc., is determined by washing a large sample of the product in a laboratory centrifugal (Fig. 185) with a saturated sugar sirup. The results obtained by a practical test of this kind are often found to have more value than those obtained by any modification of the Payen method.

**Method of Herzfeld and Zimmermann.** — In order to avoid the error due to the use of alcoholic washing fluids, Herzfeld and Zimmermann † have recently devised a method for determining crystal content, by which the raw sugar is simply shaken and washed with a saturated aqueous sucrose solution. The latter is always prepared just before use by weighing out 500 to 600 gms. of water in a strong glass-stoppered flask and adding the exact amount of sucrose to produce saturation at the laboratory temperature, which should be as near 20° C. as possible. The grams of sucrose necessary for saturating 100 gms. of water at temperatures between 15° and 35° C. are given in the following table:

| Laboratory temperature. | Grams sucrose per 100 gms. water. | Ratio of water to sugar sirup. | Laboratory temperature. | Grams sucrose per 100 gms. water. | Ratio of water to sugar sirup. |
|---|---|---|---|---|---|
| Deg. C. | | | Deg. C. | | |
| 15 | 194.3 | 2.943 | 25 | 208.3 | 3.083 |
| 16 | 195.6 | 2.956 | 26 | 209.8 | 3.098 |
| 17 | 196.9 | 2.969 | 27 | 211.3 | 3.113 |
| 18 | 198.3 | 2.983 | 28 | 212.9 | 3.129 |
| 19 | 199.6 | 2.996 | 29 | 214.5 | 3.145 |
| 20 | 201.0 | 3.010 | 30 | 216.1 | 3.161 |
| 21 | 202.4 | 3.024 | 31 | 217.7 | 3.177 |
| 22 | 203.8 | 3.038 | 32 | 219.3 | 3.193 |
| 23 | 205.3 | 3.053 | 33 | 221.0 | 3.210 |
| 24 | 206.8 | 3.068 | 34 | 222.8 | 3.228 |

The stoppered flask containing the sugar and water is warmed until the sugar has dissolved and then cooled to the required temperature. The solution is then placed in a bottle provided with a thermometer and delivery tube as shown by *F* in Fig. 186.

Fifty grams of the raw sugar are weighed into the pear-shaped glass vessel *A*, which has a capacity of about 500 c.c. and is closed at

* For a complete review of Koydl's method, with abstract of favorable and unfavorable reports, see Stammer's Jahresbericht for 1906, 1907 and 1908.

† Z. Ver. Deut. Zuckerind., **62**, 166.

its lower end by the rubber plug $S'$; 200 c.c. of the saturated sucrose solution are then added, the rubber stopper $S$ is inserted and the whole shaken vigorously until all molasses adhering to the sugar crystals has been dissolved. The vessel $A$ should not be handled with the bare hands, which might warm the solution and dissolve some of the crystals.

The vessel $A$ is then attached by the rubber stopper $B$, after removing the plug $S'$, to the filtering cup $C$. The open bottom of the latter is closed with a filter plate $h$, upon which rests a thin pad of felt $f$, and above the latter a disk of wire gauze $g$. The felt and gauze are previously cleaned, dried and weighed. The cup $C$ is then attached to the filter-flask $D$ and the stopper $S$ replaced by a stopper containing a small capillary tube. Suction is then applied and the contents of $A$ are gently discharged into $C$. The inner surface of $A$ is then washed with a little sugar solution from $F$ until all crystals are removed; about 50 c.c. of sugar solution are sufficient. The cup, without sucking off

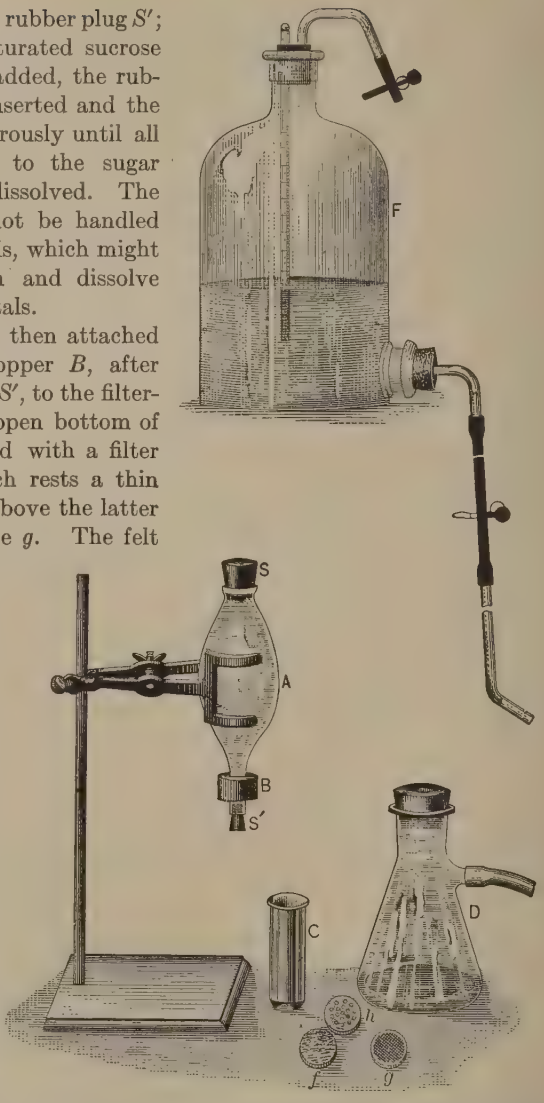



Fig. 186. — Herzfeld and Zimmermann's apparatus for determining crystal content of raw sugars.

all the sirup, is then placed in a small centrifugal and whirled for 5 minutes, in the first minute at 2000, in the second minute at 2500, and for the remaining time at 2700 revolutions per minute. The cup is then removed and its contents are discharged into a weighing bottle by inverting and gently pushing the bottom plate with a rod, any crystals left adhering to the walls of the cup being also carefully removed. The sugar, felt and gauze are weighed, and then dried in vacuum at a final temperature of 105° to 110° C. After deducting the weight of felt and gauze the loss by drying is calculated to sugar-sirup by multiplying by the ratio of water to sirup for the temperature of saturation. The weight of sirup deducted from the weight of sugar after centrifuging gives the weight of crystals. The method of calculation is illustrated by the following example:

A saturated sugar solution was prepared for a laboratory temperature of 21° C.; the ratio of water to sirup for this temperature is 1 : 3.024.

| | |
|---|---|
| Weight of raw sugar taken | = 50.00 gms. |
| Weight of sugar after centrifuging | = 48.62 gms. |
| Weight of sugar after drying | = 48.01 gms. |
| Difference due to water of sirup | = 0.61 gms. |
| Adhering sirup = 0.61 × 3.024 | = 1.84 gms. |
| Weight of crystals = 48.62 − 1.84 | = 46.78 gms. |
| Crystal content of sugar | = 93.56 per cent. |

Results of analyses of several samples of raw beet sugar by the above method are quoted from the work of Herzfeld and Zimmermann.

| Number. | Product. | Direct polarization. | Organic non-sugar. | Ash. | Moisture. | Rendement; polarization less 5 × ash. | Color degrees (Stammer) for 100 polarization. | Crystal content. | Calculated purity of molasses in raw sugar. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Raw sugar..... | 91.80 | 3.35 | 2.05 | 2.80 | 81.55 | 68 | 82.52 | 63.7 |
| | Crystals..... | 99.90 | .... | 0.11 | .... | ..... | 3.1 | ..... | .... |
| 2 | Raw sugar..... | 89.90 | 4.04 | 2.86 | 3.20 | 75.60 | 114 | 80.60 | 62.4 |
| | Crystals..... | 99.00 | .... | 0.70 | .... | ..... | 10.4 | ..... | .... |
| 3 | Raw sugar..... | 91.20 | 3.26 | 2.44 | 3.10 | 79.00 | 84 | 82.10 | 63.7 |
| | Crystals..... | 99.60 | .... | 0.37 | .... | ..... | 7.4 | ..... | .... |
| 4 | Raw sugar..... | 92.00 | 3.41 | 2.19 | 2.40 | 81.05 | 145 | 81.96 | 64.9 |
| | Crystals..... | 99.85 | .... | 0.18 | .... | ..... | 5.1 | ..... | .... |

It is seen that the final crystals obtained from the above sugars contained from 0.10 to 1.00 per cent ash and organic impurities.

The Herzfeld-Zimmermann method has not as yet been generally tested, but deserves recognition from its simplicity. The process should be subjected to a careful control according to individual technical requirements.

**Calculation of Composition and Purity of Molasses in Raw Sugars.** — A knowledge of the composition and purity of the molasses contained in raw sugars is often desired. The determination is made indirectly by subtracting the sucrose of the crystals from that of the raw sugar and calculating the remaining ingredients as due to molasses. The purity of the molasses in sugar Number 2 of the previous table would be calculated as follows:

| | Per cent. |
|---|---|
| Dry substance of raw sugar = 100.00 − 3.20 | = 96.80 |
| Crystal content of raw sugar | = 80.60 |
| Difference = Dry substance of molasses in raw sugar | = 16.20 |
| Polarization of raw sugar | = 89.90 |
| Polarization due to crystals in raw sugar = 80.60 × 0.99 | = 79.79 |
| Difference = Polarization due to molasses in raw sugar | = 10.11 |

$$\text{Apparent purity of molasses in raw sugar} = \frac{10.11}{16.20} \times 100 = 62.4$$

## Starch Products

**Polariscopic Methods for Determining Starch.** — Several methods have been devised for estimating starch from the specific rotation, after conversion into the soluble form. The following methods* have been used.

*Solution of Starch by Heating Under Pressure.* — From 2 to 3 gms. of material are heated in a 100-c.c. flask with 80 to 90 c.c. of water until a uniform gelatinization of the starch has been obtained. The flask is then placed in an autoclave (Fig. 175) and heated 3 to 5 hours at 2 to 3 atmospheres' pressure. After cooling, the clear solution is made up to 100 c.c., filtered and polarized. The soluble starch thus obtained is without action upon Fehling's solution; its rotation is $[\alpha]_D = +196.5$ to $+197$. Using the value $+196.5$, the weight of starch in the 100 c.c. of solution is calculated from the angular rotation $a$ in the 200-mm. tube by means of the formula $[\alpha]_D = \frac{100\,a}{c \times l}$, whence

$$\text{grams starch} = \frac{100\,a}{2\,(+196.5)}.$$

*Solution of Starch by Means of Hydrochloric Acid.* — Five grams of the starch-containing material are rubbed with 20 c.c. of concentrated

* Wiley's "Agricultural Analysis" (1897), Vol. III, 205.

hydrochloric acid of 1.17 sp. gr. for about 10 minutes. When the solution has cleared, the volume is completed to 200 c.c., and the liquid filtered and polarized. The soluble starch as thus prepared has a rotation of $[\alpha]_D = +196.3$ to $+196.7$. Using the mean value of $+196.5$, the grams of starch in 100 c.c. of solution are calculated as in the previous method.

With impure starch-containing materials, neither of the above polariscopic methods has the accuracy of the diastase method described on page 440.

**Calculating the Apparent Composition of Starch-conversion Products from the Specific Rotation.** — Brown, Morris and Millar* have

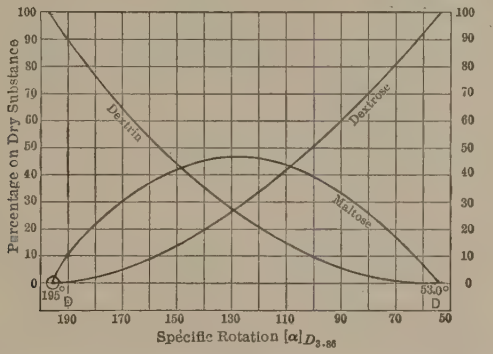



Fig. 187. — Showing relation of specific rotation to composition of acid-hydrolyzed starch products.

shown that in starch products of diastase conversion a constant relation exists between the specific rotation and copper-reducing power of the total solids. Rolfe and Defren† have also shown that in starch products of acid conversion the solids of same specific rotation have always the same reducing power "irrespective of the source of the starch, the nature or amount of the hydrolyzing acid, or the temperature conditions, these influencing the *rate* of hydrolysis only." It is, therefore, possible to express by means of a curve the relationship between specific rotation and copper-reducing power, or between either of these constants and the apparent percentages of glucose, maltose and dextrin, calculated by means of such formulæ as are used in Allen's method (p. 486). Upon this principle Rolfe has prepared the diagram shown in Fig. 187, which gives the percentages of dextrose, maltose and dextrin in the dry substance of starch-conversion products cor-

* J. Chem. Soc., **71**, 115.

† J. Am. Chem. Soc., **18**, 869; Rolfe, "The Polariscope" (1905), p. 197.

responding to the values of $[\alpha]_D$ for dry substance (as determined by the solution factor 3.86) between +195 for dextrin and +53 for glucose.

A value, for example, of $[\alpha]_D = +100$ for the dry substance (calculated from the density of an approximately 10 per cent solution at 15.5° C. by the solution factor 3.86) of an acid-conversion product would correspond to an apparent composition of dry substance of 10 per cent dextrin, 40 per cent maltose and 50 per cent glucose.

The apparent percentages as thus determined are useful for purposes of comparison and valuation but must not be mistaken for absolute percentages for reasons already given. As Rolfe is careful to state " there are comparatively few commercial products pure enough to permit of their constitution being determined in this simple manner."

**Analysis of Commercial Dextrins.** — The following method has been used by the United States Bureau of Chemistry in testing dextrins for the National Bureau of Printing and Engraving. The method is a modification by Browne and Bryan * of a scheme of analysis proposed by F. Lippmann.†

*Specific Rotation.* — Transfer 10 gms. of the finely divided sample to a 100-c.c. flask, and after solution in about 50 c.c. of cold water add 5 c.c. of alumina cream and make up the contents to 100 c.c., thoroughly shake and filter. Polarize the filtrate in a 200-mm. tube, using any form of polariscope or saccharimeter. It is important that a 6 per cent solution of bichromate of potash in a 3-mm. tube be used as a light filter. In using a Ventzke-scale saccharimeter, the specific rotation is found by the formula $[\alpha]_D = \frac{34.68 \times V}{20}$, in which $V$ = Ventzke reading.

*Viscosity.* — Dissolve 100 gms. of dextrin in 200 c.c. of cold water by rubbing up in a mortar or porcelain dish, and determine the viscosity of the solution by any of the standard forms of viscosimeter. Comparative results should always be made by the same instrument and under similar conditions of temperature; a uniform length of time should also elapse after making up the solution before taking the viscosity. The viscosity should be determined again on the same solution after standing 24 hours, and also after 48 hours.

*Moisture.* — Determine by drying from 2 to 5 gms. of sample for 4 hours at a temperature of 105° C. Absolute constancy in weight cannot be attained on account of the slow decomposition of the dextrin.

* Proc., Sec. V, " Seventh Int. Cong. App. Chem.," London, 1909, p. 337.

† Z. Spiritusind., **25**, 304, 307, 316, 317.

*Ash.* — Five to 10 gms. of the sample are weighed in a tared platinum dish and burned over a flame at a low heat. The ash should not be heated to fusion, otherwise loss from volatilization will occur.

*Soluble Starch.* — If a filtered hot-water solution of the dextrin gives a blue reaction with iodine solution, soluble starch is indicated. Weigh two lots of dextrin, 10 gms. each, into 100-c.c. flasks, add 50 c.c. of cold water to each and after all soluble matter is dissolved make up the contents of the one flask with cold water at 100 c.c., shake and filter. Evaporate 20 c.c. of the solution (2 gms.) to dryness and dry for 4 hours at 105°, as under determination of moisture. Weight of residue, less ash on incineration, equals cold-water soluble organic matter. Heat the contents of the second flask to boiling, and then after cooling make up to 100 c.c., shake and filter. The weight of hot-water soluble organic matter in 20 c.c. of solution is determined as before. Hot-water soluble organic less cold-water soluble organic gives the soluble starch.

*Unconverted Starch.* — If the residue insoluble in hot water shows under the microscope grains, which are colored blue with iodine, unconverted starch is present. To determine the percentage, collect the residue insoluble in hot water on a filter, wash until free from soluble matter, and determine the starch by the usual methods.

*Reducing Sugars.* — Determine in an aliquot of the cold-water soluble by the method of Allihn, the results being expressed as glucose.

*Dextrin.* — Subtract the specific rotation of the dextrin due to reducing sugars $\frac{(52.5 \times \text{per cent reducing sugar as glucose})}{100}$ from the original specific rotation of the sample. Multiply the remainder by 100 and divide by 186 ($[\alpha]_D$ of dextrin *) to obtain the calculated percentage of dextrin in the sample.

*Undertermined Solubles.* — The per cent of cold-water soluble organic matter less calculated percentage of dextrin gives the percentage of undetermined solubles.

In Table LXXXV eight analyses of commercial dextrins by the above method are given.

It is noted that with a decrease in specific rotation there is a uniform decrease in viscosity and in the calculated percentage of dextrin,

* The $[\alpha]_D + 186$ of dextrin is given by Schultze (J. prakt. Chem., **28,** 327). This is considerably lower than the figures +195 to +205, which have been reported by other authorities for carefully purified dextrins. The value +186 is used only as a commercial standard of comparison, and the percentages of dextrin thus calculated have no strict scientific value.

and a uniform increase in reducing sugars and undetermined matter. A large percentage of reducing sugars indicates over-dextrinization, and accompanying this there is always a formation of other decomposition products.

TABLE LXXXV

*Giving Analyses of Commercial Dextrins*

| No. | $[\alpha]_D$ | Viscosity at 20° C. 1 to 2 solution. Water = 100. | | | | Chemical analysis. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cold water solution. | | Hot-water solution. | | Moisture at 105°C | Ash. | Reducing sugars as glucose. | Cold-water insoluble organic matter. | Dextrin. | | Undetermined soluble matter. | Acidity $n/10$ KOH per 10 gms. |
| | | Immediate | After 24 hours | Immediate | After 24 hours | | | | | By difference. | From polarization. | | |
| | | | | | | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | c.c. |
| 1 | +175.2 | 844 | 1332 | 396 | 428 | 2.92 | 0.09 | 1.80 | 0.24 | 94.95 | 93.74 | 1.21 | 2.2 |
| 2 | +174.1 | 620 | 980 | 396 | 430 | 3.96 | 0.08 | 1.77 | 0.34 | 93.85 | 93.15 | 0.70 | 2.0 |
| 3 | +172.7 | 596 | 860 | 480 | 480 | 2.88 | 0.14 | 1.56 | 0.45 | 94.97 | 92.46 | 2.51 | 2.6 |
| 4 | +167.5 | 480 | 692 | 242 | 256 | 4.46 | 0.16 | 2.44 | 1.95 | 90.99 | 89.43 | 1.56 | 2.3 |
| 5 | +163.7 | 420 | 636 | 324 | 330 | 6.07 | 0.09 | 2.20 | 0.31 | 91.33 | 87.45 | 3.88 | 2.5 |
| 6 | +162.2 | 384 | 448 | 348 | 370 | 4.76 | 0.13 | 2.03 | 3.37 | 89.71 | 86.69 | 3.02 | 2.0 |
| 7 | +159.2 | 344 | 392 | 240 | 260 | 2.39 | 0.14 | 5.59 | 3.27 | 88.61 | 84.16 | 4.45 | 4.0 |
| 8 | +149.8 | 300 | 332 | 184 | 186 | 4.42 | 0.13 | 5.78 | 2.48 | 87.19 | 79.07 | 8.12 | 5.3 |

The viscosity determination is of paramount value as a physical test in examining the qualities of dextrins, likewise the change in viscosity of the cold-water solution after 24 hours' and 48 hours' standing. In the technical application of dextrins such an increase in viscosity, if large, will overtax the machines or impair the results of the work. The figures in the table corroborate the views of Lippmann that the cold-water solution only should be used for the viscosity test, since the individual differences between dextrins are thus rendered more distinguishable than where the solutions are made in hot water.

**Analysis of Malt Extracts.** — Malt extracts are employed by brewers and also by bakers, who use them extensively for the improvement of bread. The extracts are prepared by evaporating the filtered wort from mashed malt to a sirup. Malt extracts are in many cases valued for their diastatic power, and in preparing such extracts the evaporation must be conducted in a vacuum apparatus at low temperature. Extracts prepared by mashing malt with cold water have the highest diastatic activity; in such extracts the percentage of sugars preëxisting in the malt, as sucrose and invert sugar, will be high and the percentage of maltose low. If the malt be mashed at 60° C., then the extract will contain a large excess of maltose due to the conversion

of the starch by the diastase. The following analyses by Jago * show the marked difference in composition between extracts made by cold-water and warm-water mashing.

TABLE LXXXVI

| Constituent. | Cold-water mash. | | Warm-water mash, 60° C. | |
|---|---|---|---|---|
| | Extract, unevaporated. | Extract, evaporated. | Extract, unevaporated. | Extract, evaporated. |
| Water | 95.17 | 22.90 | 86.70 | 14.70 |
| Ash | 0.32 | 4.80 | 0.24 | 1.70 |
| Proteids | 0.80 | 12.71 | 0.86 | 5.27 |
| Dextrin | 0.60 | 13.66 | 1.32 | 10.82 |
| Sucrose | 0.45 | 4.79 | 0.43 | 0.00 |
| Maltose | 0.21 | 2.69 | 9.04 | 60.97 |
| Glucose and fructose | 2.45 | 38.45 | 1.41 | 6.54 |
| | 100.00 | 100.00 | 100.00 | 100.00 |

In the analysis of such a complicated mixture of sugars and carbohydrates, as occurs in malt extracts, the chemist must employ indirect methods, the use of which involves a considerable multiplication of experimental errors as previously explained (p. 488). The sucrose can be determined by the method of inversion, the dextrin by precipitation with alcohol (correcting for occluded ash and proteids), the fructose by high temperature polarization; knowing these the maltose and glucose may be calculated indirectly from the combined copper-reducing power and polarization. The results thus determined have only an approximate value. The extracts require to be clarified carefully in order to eliminate the influence of soluble proteids.

### DETERMINATION OF DIASTATIC POWER †

Malts and malt extracts are frequently purchased upon the sole basis of diastatic power and a description of several methods for determining this factor is introduced in this connection. The methods given apply also to the valuation of commercial amylases, such as diastase, takadiastase, pancreatin, etc., which find a medicinal use for certain forms of indigestion.

**Determination of Diastatic Power of Malt and Malt Extracts, Lintner's Method.** — The diastatic power of malts and malt extracts is usually determined by Lintner's‡ method, the results of which

* "The Technology of Bread Making" (1911), p. 512.

† For a fuller description of methods for determining diastatic power see Sherman's "Methods of Organic Analysis," 2nd Ed., Chapter V.

‡ J. prakt. Chem. [2], **34**, 378.

expressed as degrees Lintner, represent the copper-reducing power produced by the action of a measured volume of the extract upon a solution of soluble starch at 21° C. for 1 hour.

*Soluble-starch Solution.* — A solution is made containing 2 gms. of soluble starch (prepared as described on page 577) in 100 c.c.

*Procedure.* — In determining the diastatic power of malt, or flour, 25 gms. of the finely ground material are digested with 500 c.c. of water at room temperature for 5 hours. The solution is then filtered until perfectly clear.

Ten test tubes are placed in a metal rack and 10 c.c. of the soluble-starch solution added to each. To the first tube 0.1 c.c. of the filtered malt solution is added, to the second tube 0.2 c.c., and so on, the tenth tube receiving 1.0 c.c. The tubes are shaken and then placed for 1 hour in a water bath kept at 21° C., 5 c.c. of mixed Fehling's solution are then added to each tube and the rack is placed in a boiling-water bath for 10 minutes. The rack is then removed and, after the precipitates of cuprous oxide have settled, the two tubes are selected in which the copper is all reduced and in which some of it still remains in solution, as is shown by the absence or presence of blue color, or by means of the ferrocyanide test. The amount of malt solution just necessary to reduce the 5 c.c. of Fehling's solution is between the amounts added to these two tubes; the corrected amount is then assumed to be midway between these limits, or the value of the second decimal estimated from the depth of blue color in the tube where reduction is incomplete.

A malt is given a diastatic value of 100 on Lintner's scale when 0.1 c.c. of the filtered 5 per cent extract just reduces 5 c.c. of Fehling's solution under the above conditions. If 0.25 c.c. of malt solution were required to reduce the 5 c.c. of Fehling's solution then the diastatic power of the malt would be $\frac{0.1 \times 100}{0.25} = 40$ degrees Lintner. A slight correction remains to be made for the reducing sugars in the malt solution and for any reducing power of the soluble starch. This correction is found by taking 5 c.c. of Fehling's solution, 10 c.c. of starch solution and 10 c.c. of water and heating to boiling. The malt solution is then added from a burette until the blue color is just discharged. If 7 c.c. of malt solution were used then there would be a correction of $\frac{0.1 \times 100}{7} = 1.4$ degrees Lintner to be subtracted from the value previously found.

In the case of evaporated malt extracts of high diastatic power a 1 per cent or 0.5 per cent solution of the extract is used, the values thus

obtained being multiplied by 5 or 10 to obtain the true degrees Lintner for a 5 per cent solution.

**Lintner's Method as Applied to Diastases.** — In determining the activity of diastase preparations Lintner * uses the method described for malt, the only difference being that the results are expressed in terms of a diastase of which 0.12 mg. produces sufficient sugar to reduce the 5 c.c. of Fehling's solution. In making the test, from 50 to 100 mgs. of the diastase to be tested are dissolved in 4 to 5 c.c. of water and then made up to 100 c.c. or 200 c.c. according to the supposed strength of the enzyme. If under the conditions described for the malt method 0.2 mg. of a diastase was required to produce sufficient sugar to reduce the 5 c.c. of Fehling's solution, then its diastatic power would be $\frac{0.12 \times 100}{0.2} = 60$ degrees Lintner (diastase scale).

It should be noted that 100 degrees *diastase* are over 40 times $\left(\frac{5.0 \text{ mgs.}}{0.12 \text{ mgs.}}\right)$ as powerful as 100 degrees *malt* upon Lintner's scale.

**Sykes and Mitchell's Gravimetric Modification of Lintner's Method.** — In the method of Sykes and Mitchell † 100 c.c. of 2 per cent soluble-starch solution are treated with 1 c.c. of the 5 per cent malt extract (prepared as in Lintner's method) at 21° C. for 1 hour; 50 c.c. of Fehling's solution are then added and the liquid heated quickly to 98° C., when it is placed in a boiling-water bath for 7 minutes. The reduced copper is then determined, the weight of which divided by 0.438 (the grams of copper in 50 c.c. Fehling's solution) and multiplied by 100 gives the diastatic power in degrees of the Lintner scale. The results are said to compare well with those obtained by Lintner's method.

A gravimetric method for determining diastatic power permits a closer degree of estimation than is possible by the original Lintner process. Slight errors of estimation by the volumetric method cause considerable differences in the final results, when only small volumes of diastase solution are taken. Thus between 0.1 c.c. and 0.15 c.c. the degrees Lintner (malt) will vary between 100 and 66.6.

**Determination of Diastatic Power of Commercial Amylases, Method of Sherman, Kendall and Clark.** ‡ — In studying methods for determining the diastatic power of commercial pancreatin, Sherman, Kendall and Clark found that the conditions of temperature and

* J. prakt. Chem. [2], **34,** 378; **36,** 481.
† Analyst, **21,** 122.
‡ J. Am. Chem. Soc., **32,** 1073.

activation under which an amylase normally works should be incorporated in the method. These authorities also showed that the amount of reduced copper does not stand in simple proportion to diastatic power, different diastatic values being obtained when different weights of enzyme were taken. These differences are due to the influence of variations in the concentration of starch upon the rate of conversion; if the velocity of the reaction be considered, however, the same diastatic power is derived from the weight of reduced copper for any weight of enzyme. The following gravimetric method was used.

*Enzyme.* — The enzyme may be dissolved in pure water if its power is to be tested immediately. If it is to stand, it should be dissolved in water containing 4 c.c. of fiftieth-molar disodium phosphate per 100 c.c. The test should be made within an hour in any case. The amount of enzyme to be weighed out will depend entirely on its strength.

*Activating Agents.* — These will doubtless differ with the different amylases. For pancreatic amylase acting on 2 per cent starch, add 300 mgs. sodium chloride and 7 c.c. of fiftieth-molar disodium phosphate per 100 c.c. (final volume) of reaction mixture.

*Procedure.* — Prepare 400 c.c. of 2 per cent soluble-starch solution and the enzyme solution of such a strength that 1 c.c. will contain from 0.4 to 1.0 mg. of enzyme. By means of a 1 c.c. Mohr's pipette, accurately calibrated in hundredths, measure into four 200 c.c. Erlenmeyer flasks such volumes of the solution as will contain 0.2, 0.5, 0.8 and 1.0 mg. of enzyme, respectively. Now 100 c.c. of the starch solution, previously warmed to 40° C. is poured into each flask and the digestion allowed to proceed for 30 minutes, the temperature being accurately maintained at 40° C. At the expiration of the 30 minutes, stop the reaction quickly by mixing at once with 50 c.c. of Fehling's solution and immerse the flask in a large bath of boiling water for 15 minutes. See that the water of the bath is kept boiling and that it stands above the level of the contents of any of the flasks. At the end of this heating filter quickly and determine the reduced copper by any accurate method.

Correct the weight of reduced copper or cuprous oxide found for the reducing power of the soluble starch by subtracting from it the weight obtained in a "blank" test in which the starch solution is treated directly with the Fehling reagent. Of the four determinations thus corrected, select the highest weight of cuprous oxide which does not exceed 300 mgs. and find the corresponding value of $K$ in the following table. This value of $K$ divided by the milligrams of substance gives the diastatic power of the enzyme upon Sherman's scale.

*Values for K from Cuprous Oxide Found*

| Cuprous oxide. | *K.* | Cuprous oxide. | *K.* | Cuprous oxide. | *K.* | Cuprous oxide. | *K.* |
|---|---|---|---|---|---|---|---|
| Mgs. | | Mgs. | | Mgs. | | Mgs. | |
| 30 | 9.1 | 100 | 31.2 | 170 | 54.1 | 240 | 78.3 |
| 40 | 12.2 | 110 | 34.4 | 180 | 57.5 | 250 | 81.8 |
| 50 | 15.3 | 120 | 37.6 | 190 | 60.9 | 260 | 85.4 |
| 60 | 18.4 | 130 | 40.9 | 200 | 64.3 | 270 | 89.0 |
| 70 | 21.6 | 140 | 44.2 | 210 | 67.8 | 280 | 92.6 |
| 80 | 24.8 | 150 | 47.5 | 220 | 71.3 | 290 | 96.3 |
| 90 | 28.0 | 160 | 50.8 | 230 | 74.8 | 300 | 100.0 |

*Example.* — A sample of soluble starch which had been treated with 1.5 mgs. of enzyme gave 295.5 mgs. of cuprous oxide; the blank test for the soluble starch gave 60.5 mgs. The corrected weight of cuprous oxide is $295.5 - 60.5 = 235$ mgs. which corresponds to a value for $K$ of 76.6. $\frac{76.6}{1.5} = 51$, the diastatic power of the enzyme by Sherman's scale.

The values for $K$ in the above table represent the rate of diastatic conversion and were determined by means of a velocity curve which was plotted with different periods of time as abscissæ and different yields of cuprous oxide as ordinates (see p. 695).

**Iodine Method for Determining Diastatic Power.** — A number of methods have been devised for determining diastatic power colorimetrically by means of iodine. In Wohlgemuth's* method several 5 c.c. portions of a 1 per cent solution of soluble starch are treated with different amounts of diastase at 40° C. for 30 minutes. The solutions after diluting to a measured volume are then treated with 1 drop of $n/10$ solution of iodine and, after shaking, the tube selected in which the deep blue and violet of soluble starch have given place to the red or orange red of erythrodextrin. The amount of enzyme added to this tube is noted and its diastatic power calculated as the number of cubic centimeters of 1 per cent soluble-starch solution which would be converted by 1 c.c. or 1 gm. of substance. Thus if 0.02 c.c. of saliva converts 5 c.c. of 1 per cent soluble-starch solution in 30 minutes at 40° C. 1 c.c. will digest 250 c.c. The diastatic power of the saliva is then expressed as $D_{30'}^{40°} = 250$ (Wohlgemuth's scale).

The diastatic values obtained by the iodine method represent the dextrinizing rather than the saccharifying power of an amylase. For certain physiological purposes the results of the iodine method may have a greater value although the difficulty of securing a satisfactory end point interferes at times with the accuracy of the method.

* Biochem. Zeitschr., **9**, 1–9.

## Miscellaneous Food Products

The detection and estimation of sugars in food products are made according to the physical and chemical methods previously described. Such methods are often valueless, however, for many purposes of the food chemist, who frequently desires to know more about the origin of the sugars in his product than about their nature or exact amount. A polarization of maple sugar, for example, will not determine whether its sucrose was derived from the maple or sugar cane. Neither does an estimation of the invert sugar and dextrin in a honey determine whether these have been gathered by the bee or have been added as an adulteration. In the solution of such problems as these the food chemist must base his decision upon reactions and estimations of other ingredients than sugar, such, for example, as the amount of matter precipitated by lead subacetate or by alcohol, the composition of the ash and organic non-sugars, miscroscopical examination, etc. Such determinations lie strictly outside the province of sugar analysis and only a few typical applications of such methods will be considered. For a fuller description of such processes the chemist is referred to the special works upon food analysis by Leach, Wiley, Allen, Blythe, König and others.

### Determination of Lead-Subacetate Precipitate

The determination of the amount of lead-subacetate precipitate is frequently used as a means of distinguishing pure maple sugars and sirups from those which are adulterated with cane sugar. The method is based upon the presence in maple products, and the absence in cane sugars, of salts of malic acid which gives a copious precipitate with lead subacetate.

**Hortvet's* Method for Measuring the Volume of Lead Precipitate.** — *Apparatus.* — The apparatus consists of a glass tube and holder as shown in Fig. 188. The tube and holder weigh about 50 gms., and should be so constructed that when fitted together the bottom of the tube will be exactly even with the lower surface of the holder. In a set, each couple, tube and holder should be made to balance one another. When placed in the centrifuge there should be as nearly as possible a balanced load carried at the circumference of the wheel.

*Determination.* — Introduce into the tube 5 c.c. of sirup or 5 gms. of sugar, add 10 c.c. of water and dissolve. Add 0.5 c.c. (10 drops) of alumina cream (prepared as directed on page 223) and 1.5 c.c. of lead sub-

* J. Am. Chem. Soc., **26**, 1523.

acetate and shake thoroughly. Allow the mixture to stand from 45 to 60 minutes, occasionally giving the tube a twisting motion to facilitate the settling of the precipitate. Place the tube with its holder in the centrifugal machine and run 6 minutes under the conditions given below. If any material adheres to the sides of the wider portion, remove it by means of a small wire provided with a loop at the end. Return the tube to the centrifuge and run 6 minutes longer at the same rate. Note the volume of the precipitate, estimating to 0.01 c.c. as closely as possible. Run a blank, using water and the reagents named above, and correct for same. In the case of a sirup the result is reduced to the 5-gm. basis by dividing by the specific gravity of the sample.

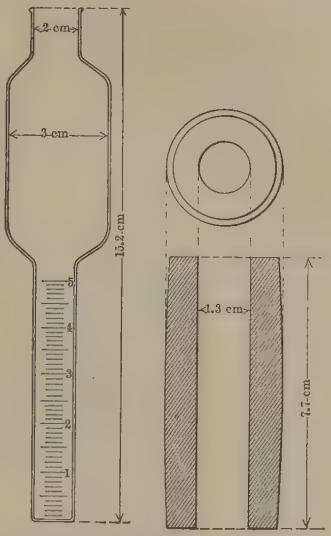



Fig. 188. — Hortvet's apparatus for measuring volume of lead precipitate.

The centrifuge used in this method has a radius of 18.5 cm. and is run at a speed of 1,600 revolutions per minute. The velocity at the circumference of the wheel is computed in centimeters per second. Calling $M$ (mass) unity in the formula $F = \frac{Mv^2}{r}$, the numerical expression for $F$, the centrifugal force, becomes 519,363.

By measuring the radius ($r$) for any given machine and substituting for $F$, the numerical constant determination above, the velocity for a given machine may be determined by the following formula, $v = \sqrt{Fr}$. Given the velocity in centimeters per second, the required number of revolutions per second or per minute can be computed.

The volume of lead precipitate, as determined above, was found by Hortvet to vary from 0.94 c.c. to 1.82 c.c. for pure maple sirups, and from 1.18 c.c. to 4.41 c.c. for pure maple sugars. Adulterated maple sirups gave from 0.23 c.c. to 0.95 c.c. and adulterated maple sugars from 0.10 c.c. to 1.40 c.c.

**Winton's* Method for Determining Precipitated Lead (Lead Number).** — Weigh 25 gms. of the material (or 26 gms. if a portion of

* J. Am. Chem. Soc., **28,** 1204.

the filtrate is to be used for polarization) and transfer by means of boiled water into a 100-c.c. flask. Add 25 c.c. of standard lead-subacetate solution, fill to the mark, shake, allow to stand at least 3 hours and filter through a dry filter. From the clear filtrate pipette off 10 c.c., dilute to 50 c.c., add a moderate excess of sulphuric acid and 100 c.c. of 95 per cent alcohol. Let stand over night, filter on a Gooch crucible, wash with 95 per cent alcohol, dry at a moderate heat, ignite at low redness for 3 minutes, taking care to avoid the reducing cone of the flame, cool and weigh. Calculate the amount of lead in the precipitate using the factor 0.6831, subtract this from the amount of lead in 2.5 c.c. of the standard solution, multiply the remainder by 100 and divide by 2.5, thus obtaining the lead number.

The standard lead-subacetate is prepared by diluting a measured volume of lead-subacetate reagent of 1.25 sp. gr. with 4 volumes of water, and filtering if not perfectly clear.

The lead number, as determined above, was found by Winton and Kreider to vary from 1.19 to 1.66 for pure maple sirups, and from 1.83 to 2.48 for pure maple sugar. Adulterated maple sirups gave lead numbers ranging from 0.02 to 0.92.

**Limitations of the Lead-precipitate Methods.** — Raw cane sugars (especially such as are made without clarification and hence contain all the organic salts of the juice) may give amounts of lead precipitate which are as great as those obtained with pure maple products. Doolittle and Seeker * give, for example, the following comparison between a Venezuelan muscovado sugar ("Melada") and a pure Vermont maple sugar.

TABLE LXXXVII

| Determination. | Muscovado sugar. | Vermont maple sugar. |
|---|---|---|
| Moisture (per cent) | 7.50 | 2.80 |
| Ash (per cent) | 1.30 | 1.10 |
| Polarization, direct at room temperature (° V.) | +82.4 | +84.0 |
| Polarization, invert at room temperature (° V.) | −26.8 | −29.6 |
| Invert polarization, at 86° (° V.) | ± 0.0 | ± 0.0 |
| Sucrose (Clerget) (per cent) | 83.1 | 85.6 |
| Winton lead number | 2.12 | 2.26 |

It is seen from the above that the polarization and lead number are not always sufficient to distinguish between cane and maple sugar. The results of the lead-precipitate method should always be confirmed by other means.

* Bull., 122, U. S. Bur. of Chem., p. 196.

### ANALYSIS OF ASH AS A MEANS OF DETERMINING THE ORIGIN OF SUGARS

One of the most valuable methods of ascertaining the source of a sugar is to determine the composition of its ash. The mineral constituents of the juice of the maple, sugar beet and sugar cane show very pronounced differences, and, notwithstanding the influences of clarification and crystallization, certain of these constituents find their way into the raw sugar in sufficient quantities to afford a valuable basis of opinion. Sugar-beet juice, for example, in distinction from that of the cane and maple, contains considerable potassium nitrate and perceptible quantities of the latter are usually present in raw beet sugar. Even the higher grades of beet sugar will frequently respond to delicate tests for nitrates and this has been used as one means of distinguishing beet from cane sugar.

As an example of the application of the ash-analysis method the following results by Doolittle and Seeker* upon the muscovado and maple sugar of Table LXXXVII are given. Average determinations made by Jones † upon the ash of pure maple sugars are also added for comparison.

TABLE LXXXVIII

*Analysis of the Ash of Muscovado and Maple Sugar*

| Determination. | Muscovado sugar. | Vermont maple sugar. | Average maple sugar ash, by Jones. |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| Insoluble in boiling nitric acid (1 : 3) | 3.41 | 8.9 | ..... |
| Potassium oxide | 49.89 | 23.6 | 26.49 |
| Sodium oxide | 2.32 | 1.6 | ..... |
| Calcium oxide | 5.66 | 35.9 | 24.98 |
| Magnesium oxide | 2.63 | 3.0 | ..... |
| Ferric oxide | 0.26 | {Slight / trace} | ..... |
| Chlorine | 1.34 | Trace | ..... |
| Sulphur trioxide | 23.21 | None | 1.82 |
| Phosphoric acid | 3.68 | 0.45 | ..... |
| Undetermined | 7.60 | 26.55 | ..... |
| Water-soluble ash (per cent) | 1.23 | 0.50 | 0.53 |
| Water-insoluble ash (per cent) | 0.17 | 0.64 | 0.48 |
| Ratio $\frac{\text{water-soluble ash}}{\text{water-insoluble ash}}$ | 7.7 | 0.8 | 1.1 |
| Alkalinity of water-soluble ash (c.c. tenth-normal acid per ash of 1 gm. of sample) | 0.11 | 0.49 | 0.68 |
| Alkalinity of water-insoluble ash (c.c. tenth-normal acid per ash of 1 gm. of sample) | 0.03 | 1.47 | 1.01 |

* Bull., 122, U. S. Bur. of Chem., p. 196.

† Eighteenth Annual Report, Vermont Agr. Exp. Sta. (1905), p. 331.

It is seen that in certain constituents, as potassium oxide, calcium oxide, and sulphur trioxide, the ashes of the muscovado and maple sugars show very pronounced differences. The determinations of water-soluble and water-insoluble ash and of the alkalinities of the latter are valuable aids in forming an opinion as to the origin of a sugar. The ash for such determinations should be prepared according to the method described for quantitative examination (page 495).

### DETERMINATION OF ALCOHOL PRECIPITATE

The determination of the amount of substance precipitated by strong alcohol is frequently used in examining sugar-containing products. The materials which are precipitated by alcohol may consist of mineral or organic salts, pectin, dextrin, dextran and other gums. In many cases a qualitative examination of the alcohol precipitate throws considerable light upon the origin of the product.

**Determination of Alcoholic Precipitate in Fruit Products.** *Method of the Association of Official Agricultural Chemists.** — Evaporate 100 c.c. of a 20 per cent solution of the fruit product to 20 c.c.; add slowly and with constant stirring 200 c.c. of 95 per cent alcohol and allow the mixture to stand over night. Filter and wash with 80 per cent alcohol by volume. Wash this precipitate off the filter paper with hot water into a platinum dish, evaporate to dryness, dry at 100° C. for several hours and weigh; then burn off the organic matter and weigh the residue as ash. The loss in weight upon ignition is called alcohol precipitate.

The ash should be largely lime and not more than 5 per cent of the total weight of the alcohol precipitate. If it is larger than this some of the salts of the organic acids have been brought down. Titrate the water-soluble portion of this ash with tenth-normal acid, as any potassium bitartrate precipitated by the alcohol can thus be estimated.

The general appearance of the alcohol precipitate is one of the best indications as to the presence of glucose and dextrin. Upon the addition of alcohol to a pure fruit product a flocculent precipitate is formed with no turbidity, while in the presence of glucose a white turbidity appears at once upon adding the alcohol, and a thick gummy precipitate forms.

When the quantity of gum or dextrin is large, a considerable amount of sugar is sometimes occluded in the alcohol precipitate. This is especially the case with honey, for determining the dextrin in which Browne has modified the alcohol precipitate method as follows.

* Bull. 107 (revised), U. S. Bur. of Chem., p. 80.

**Determination of Alcohol Precipitate in Honey.** *Browne's* * *Method.* — Eight grams of honey are transferred to a 100-c.c. flask with 4 c.c. of water and sufficient absolute alcohol to complete to the mark. A little care is required to effect the complete removal of the honey from the weighing dish without using more than 4 c.c. of water. The transference is best made by decanting as much as possible of the liquefied honey into the flask, then adding 2 c.c. of water to the dish to take up any adhering honey and again decanting. By using 1 c.c. more of the water in two successive washings and adding a few cubic centimeters of the absolute alcohol each time before decanting, the honey can be completely transferred without the necessity of using more water than the 4 c.c. Absolute alcohol is used finally to rinse out the dish and is then added to the flask with continual agitation until the volume is completed to 100 c.c. After shaking thoroughly the flask is allowed to stand until the dextrin has settled out upon the sides and bottom and the supernatant liquid has become perfectly clear (usually in 24 hours).

The clear solution is then decanted through a filter and the precipitated residue washed with 10 c.c. of cold 95 per cent alcohol to remove adhering liquid, the washings being also poured through the filter. The residue adhering to the flask and the particles which may have been caught upon the filter are dissolved in a little boiling distilled water and washed into a weighed platinum dish. The contents of the latter are then evaporated and dried in a water oven to constancy in weight. Should the amount of precipitate be considerable, it is necessary to dry upon sand in vacuo at 70° C.

After determining the weight of the dried alcohol precipitate the latter is redissolved in water and made to a definite volume. The following dilutions are employed in making up the solutions:

| Weight of precipitate in grams | 0.0–0.5 | 0.5–1.0 | 1.0–1.5 | 1.5–2.0 | 2.0–2.5 | 2.5–3.0 |
|---|---|---|---|---|---|---|
| Volume of solution in cubic centimeters | 50 | 100 | 150 | 200 | 250 | 300 |

The sugars are then determined in aliquots from the filtered solution of alcohol precipitate both before and after inversion. The total precipitate less invert sugar and sucrose gives the per cent of dextrin.†

While this method of estimating dextrin in honeys gives much more accurate results than the direct weighing of the alcohol pre-

* Bull. 110, U. S. Bur. of Chem., p. 19.

† With honeydew honey, which gives a large amount of alcohol precipitate, it is found best to take only 4 gms. of honey for analysis; in other respects the method of procedure is the same.

cipitate, it can not be said in any way to give the true dextrin content of the honey, although it is believed that the figures obtained are a close approximation. A small amount of dextrin always escapes precipitation with alcohol; furthermore no account is taken of those ingredients which may be occluded in the alcohol precipitate other than the sugars, and no correction is made for the copper-reducing power of the honey dextrin itself. This latter factor, though apparently very small, might prove to be of some importance if much dextrin were present. Notwithstanding these limitations, however, the percentage of dextrin as determined by the method described has been found to have a decided value, especially when it is wished to compare honeys of different origins.

The percentages of dextrin in different American honeys, as determined by the above method, is given in the following table of composition, which is taken from the work of Browne. The honeys are arranged in order of their dextrin content.

TABLE LXXXIX

*Giving Composition of American Honeys. Bull. 110, U. S. Bur. of Chem.*

| Kind of honey. | Number of samples | Polarization 20° C. | Water. | Invert sugar. | Sucrose | Ash. | Dextrin. | Undetermined. |
|---|---|---|---|---|---|---|---|---|
| | | Deg. V. | Per cent | Per cent | Per cent | Per cent | Per cent | Per cent |
| Alfalfa | 8 | −15.10 | 16.56 | 76.90 | 4.42 | 0.07 | 0.34 | 1.71 |
| Apple | 2 | − 8.55 | 15.67 | 73.16 | 3.69 | 0.08 | 0.39 | 7.01 |
| Orange | 1 | −15.50 | 16.99 | 77.57 | 0.60 | 0.08 | 0.45 | 4.31 |
| Sweet clover | 4 | −17.61 | 17.49 | 76.20 | 2.24 | 0.12 | 0.45 | 3.50 |
| Raspberry | 2 | −18.85 | 18.08 | 74.52 | 1.42 | 0.05 | 0.56 | 5.37 |
| Mangrove | 2 | −22.80 | 19.18 | 76.49 | 1.73 | 0.20 | 0.56 | 1.84 |
| White clover | 15 | −13.01 | 17.64 | 74.92 | 1.77 | 0.07 | 0.82 | 4.78 |
| Cotton | 2 | −17.50 | 18.35 | 75.43 | 1.38 | 0.21 | 1.10 | 3.53 |
| Buckwheat | 2 | −16.80 | 18.54 | 76.85 | 0.03 | 0.09 | 1.22 | 3.27 |
| Dandelion | 2 | −12.40 | 14.54 | 76.84 | 3.12 | 0.16 | 1.23 | 4.11 |
| Tupelo | 2 | −24.00 | 17.34 | 72.24 | 3.01 | 0.07 | 2.08 | 5.26 |
| Golden rod | 3 | −12.33 | 19.88 | 72.02 | 1.68 | 0.16 | 2.18 | 4.08 |
| Willow | 1 | −12.80 | 19.11 | 71.47 | 0.95 | 0.35 | 2.75 | 5.37 |
| Basswood | 6 | − 8.90 | 17.42 | 75.14 | 0.72 | 0.20 | 3.07 | 3.45 |
| Sumac | 3 | −10.47 | 18.85 | 71.11 | 0.92 | 0.44 | 3.57 | 5.11 |
| Yellow wood | 1 | − 7.00 | 18.12 | 71.51 | 0.19 | 0.39 | 4.10 | 5.69 |
| White wood | 1 | − 4.90 | 17.47 | 69.02 | 2.72 | 0.51 | 5.59 | 4.69 |
| Poplar | 1 | + 3.60 | 17.02 | 65.80 | 3.10 | 0.76 | 10.19 | 3.13 |
| White oak | 1 | +11.00 | 13.56 | 65.87 | 4.31 | 0.79 | 10.49 | 4.98 |
| Hickory | 1 | + 7.80 | 16.05 | 65.89 | 2.76 | 0.78 | 12.95 | 1.57 |

The dextrins of honey are derived largely from *honeydew* (the gummy exudation from leaves, buds, etc.) and not from floral nectar. Honeydew contains considerable mineral matter, and its presence in honey causes a marked increase in the ash content. Honey dextrin is

strongly dextrorotatory ($[\alpha]_D$ varies from about +115 to +160) and the presence of much honeydew may cause honey to polarize to the right.

If commercial glucose is suspected, honeydew dextrins may be distinguished from those of starch conversion by dissolving the alcohol precipitate in a little water and adding a few cubic centimeters of iodine solution; a red color, due to erythrodextrin, indicates the presence of commercial glucose.

# PART II

## THE OCCURRENCE, METHODS OF PREPARATION, PROPERTIES AND PRINCIPAL REACTIONS OF THE SUGARS AND ALLIED DERIVATIVES

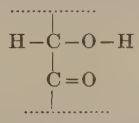
# CHAPTER XVIII

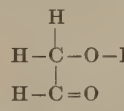
## CLASSIFICATION OF THE SUGARS AND THEIR FORMATION IN NATURE

The sugars, of which some thirty or more have been isolated from plant and animal substances, are among the most widely distributed organic compounds in nature.

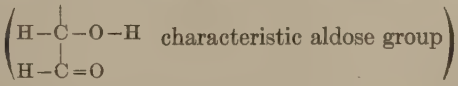
The sugars proper, including the monosaccharides, disaccharides, trisaccharides and tetrasaccharides, are colorless, odorless, crystalline substances, usually of sweet taste, and for the most part easily soluble in water. The more complex anhydride condensation products of the sugars, the polysaccharides, are usually amorphous compounds of little or no solubility in water. The entire group of saccharides, the so-called carbohydrates, constitute approximately three-fourths of the dry matter of the plant world.

**The Simple Sugars.** — A simple sugar, or monosaccharide, may be defined as an aldehyde or ketone alcohol of the aliphatic series, the molecule of which contains one carbonyl and one or more alcohol groups, one of the latter being always adjacent to the carbonyl group.

All sugars contain, therefore,  as a characteristic group upon the presence of which nearly all of the chemical properties of the sugars depend. The simplest possible sugar according to the above is

glycol aldehyde. 

Sugars containing the aldehyde group are termed aldoses



and those containing the ketone group ketoses

According to the number of their carbon atoms the monosaccharides are divided into dioses ($C_2H_4O_2$), trioses ($C_3H_6O_3$), tetroses ($C_4H_8O_4$), pentoses ($C_5H_{10}O_5$), hexoses ($C_6H_{12}O_6$), heptoses ($C_7H_{14}O_7$), octoses ($C_8H_{16}O_6$) and nonoses ($C_9H_{18}O_9$). There are also substituted monosaccharides in which one or more hydrogen atoms of a diose, triose, tetrose, etc., are replaced by a methyl group, as, for example, methyldiose ($CH_3C_2H_3O_2$), dimethyldiose ($CH_3C_2H_2O_2CH_3$), methyltriose ($CH_3C_3H_5O_3$), methyltetrose ($CH_3C_4H_7O_4$), methylpentose ($CH_3C_5H_9O_5$), methylhexose ($CH_3C_6H_{11}O_6$), methylheptose ($CH_3C_7H_{13}O_7$), etc.

**The Compound Sugars.** — By the condensation of 2, 3 or 4 molecules of the monosaccharides, the disaccharides, trisaccharides and tetrasaccharides are formed. In such condensations one molecule less of water is eliminated than the number of reacting sugars, thus:

$$2\,C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11} \text{ (disaccharide).}$$
$$3\,C_6H_{12}O_6 - 2\,H_2O = C_{18}H_{32}O_{16} \text{ (trisaccharide).}$$
$$4\,C_6H_{12}O_6 - 3\,H_2O = C_{24}H_{42}O_{21} \text{ (tetrasaccharide).}$$

**The Polysaccharides.** — By the condensation of an indefinite number of molecules of the monosaccharides the polysaccharides are formed. In such condensations one molecule less of water is probably eliminated than the total number of reacting sugar molecules, as, for example:

$$\underset{\text{Pentose}}{n\,C_5H_{10}O_5} - (n-1)\,H_2O = \underset{\text{Pentosan.}}{(C_5H_8O_4)_nH_2O.}$$
$$\underset{\text{Hexose}}{n\,C_6H_{12}O_6} - (n-1)\,H_2O = \underset{\text{Hexosan.}}{(C_6H_{10}O_5)_nH_2O.}$$

The quantity $n$ is usually so large, however, that the formulæ of the polysaccharides may be taken as simply $(C_5H_8O_4)_n$, $(C_6H_{10}O_5)_n$, etc., without sensible error.

**Carbohydrates.** — The term carbohydrate is a general one which is frequently applied to the entire group of saccharides. In its original sense it was applied only to such saccharide substances as contain six, or a multiple of six, carbon atoms and have their hydrogen and oxygen in the proportion to form water. Such substances were regarded loosely as simple compounds of carbon and water, and hence the name carbohydrate. Thus:

$$\text{Glucose, } C_6H_{12}O_6 = 6\,C + 6\,H_2O.$$
$$\text{Sucrose, } C_{12}H_{22}O_{11} = 12\,C + 11\,H_2O.$$
$$\text{Raffinose, } C_{18}H_{32}O_{16} = 18\,C + 16\,H_2O.$$
$$\text{Cellulose, } (C_6H_{10}O_5)_n = n\,(6\,C + 5\,H_2O).$$

This original meaning of carbohydrate is still retained by some writers, although it was proved long ago that the term can no longer be

taken in its former literal sense. A large number of sugars contain less than six, or a fractional multiple of six, carbon atoms, and there are also many sugars whose hydrogen and oxygen atoms have a different ratio than in water, such, for example, as the methylpentoses, $C_6H_{12}O_5$.

**Alcohol and Acid Derivatives of Sugars.** — The term carbohydrate is very often extended to include the alcohol and acid derivatives of the simple sugars. While this extension of meaning is not approved of by all chemists, a knowledge of these compounds so closely allied to the sugars is indispensable. The monosaccharides, as aldehydes, stand midway between the alcohols and acids. They are easily reduced to the former on the one hand and readily oxidized to the latter on the other. Such reactions take place continually in the chemical processes of plant and animal life, and also occur in the industrial operations of sugar factories, distilleries, etc. A proper understanding of this relationship is, therefore, of great importance. The following table, which gives a classification of the alcohols, sugars and acids of different monosaccharides, will make the mutual relationship of these more clear. The members, which are found in nature either free or in a polysaccharide form, are printed in heavy type.

TABLE XC

*Showing Group Relationships of Alcohols, Sugars and Acids*

| Group. | Alcohol. | Sugar. | Monobasic acid. | Dibasic acid. |
|---|---|---|---|---|
| Diose (aldose) | **Glycol** $\begin{matrix} H \\ H-C-OH \\ \vert \\ H-C-OH \\ H \end{matrix}$ | Glycolose $\begin{matrix} H \\ H-C-OH \\ \vert \\ H-C=O \end{matrix}$ | **Glycollic** $\begin{matrix} H \\ H-C-OH \\ \vert \\ HO-C=O \end{matrix}$ | **Oxalic** $\begin{matrix} HO-C=O \\ \vert \\ HO-C=O \end{matrix}$ |
| Methyldiose (aldose) | **Methylglycol** $CH_3-C_2H_5O_2$ | Methylglycolose $CH_3-C_2H_3O_2$ | **Lactic** $CH_3-C_2H_3O_3$ | |
| Dimethyldiose (ketose) | Dimethylglycol $CH_3-C_2H_4O_2-CH_3$ | **Dimethylglycolose** $CH_3-C_2H_2O_2-CH_3$ | | |
| Triose (aldose) | **Glycerol** $C_3H_8O_3$ | Glycerose $C_3H_6O_3$ | Glyceric $C_3H_6O_4$ | Tartronic $C_3H_4O_5$ |
| Tetrose (aldose) | **Erythrite** $C_4H_{10}O_4$ | Erythrose $C_4H_8O_4$ | Erythronic $C_4H_8O_5$ | **Tartaric** $C_4H_6O_6$ |
| Pentose (aldose) | Arabite<br>Xylite<br>**Adonite**<br>$C_5H_{12}O_5$ | **Arabinose**<br>**Xylose**<br>Ribose<br>$C_5H_{10}O_5$ | Arabonic<br>Xylonic<br>Ribonic<br>$C_5H_{10}O_6$ | Trioxyglutaric<br>Xylotrioxyglutaric<br>Ribotrioxyglutaric<br>$C_5H_8O_7$ |
| Methylpentose (aldose) | Rhamnite<br>Fucite<br>Rhodeite<br>$C_6H_{14}O_5$ | **Rhamnose**<br>**Fucose**<br>**Rhodeose**<br>$C_6H_{12}O_5$ | Rhamnonic<br>Fuconic<br>Rhodeonic<br>$C_6H_{12}O_6$ | |

TABLE XC (*Continued*)
*Showing Group Relationships of Alcohols, Sugars and Acids*

| Group. | Alcohol. | Sugar. | Monobasic acid. | Dibasic acid. |
|---|---|---|---|---|
| Hexose (aldose) | **Sorbite**<br>**Mannite**<br>**Dulcite**<br>$C_6H_{14}O_6$ | **Glucose**<br>**Mannose**<br>**Galactose**<br>$C_6H_{12}O_6$ | Gluconic<br>Mannonic<br>Galactonic<br>$C_6H_{12}O_7$ | Saccharic<br>Mannosaccharic<br>Mucic<br>$C_6H_{10}O_8$ |
| Hexose (ketose) | Sorbite+Mannite<br>Sorbite+Idite<br>$C_6H_{14}O_6$ | **Fructose**<br>**Sorbose**<br>$C_6H_{12}O_6$ | | |
| Heptose | **Perseite**<br>**Volemite**<br>$C_7H_{16}O_7$ | Mannoheptose<br>Volemose<br>$C_7H_{14}O_7$ | Mannoheptonic<br>$C_7H_{14}O_8$ | Pentoxypimelic<br>$C_7H_{12}O_9$ |

**The Asymmetric Carbon Atom and the Optical Activity of Sugars.** — As first pointed out by Van't Hoff * and Le Bel † the optical activity of sugars, as of other organic substances, is associated with the presence of an asymmetric carbon atom, by which is meant a carbon atom united to four dissimilar atoms or groups. Upon inspecting the structural formula of glycolose in the preceding table it is seen that two valences of one C atom are united alike to two H atoms, and that two valences of the other C atom are united alike to an O atom. Glycolose contains no asymmetric carbon atom and must, therefore, be optically inactive.

In the sugar glycerose, on the other hand, the central C atom is united with the four dissimilar atoms or groups, $CH_2OH$, H, OH and

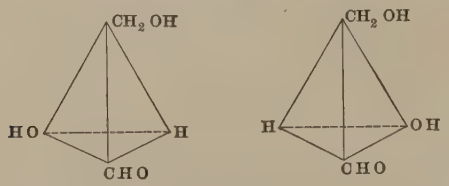



Fig. 189. — Models illustrating antipodal forms of glycerose.

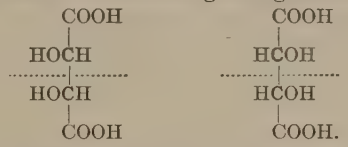
CHO; glycerose must, therefore, exist in an optically active form. If the four groups connected with the asymmetric C atom of glycerose be placed at the points of a tetrahedral model, as in Fig. 189, it will be found that two structural combinations alone are possible. These two forms, which bear the relationship of mirror images to each other, cannot by any manner of turning be superimposed. They constitute a pair of optical isomers, or antipodes, one of which is dextrorotatory and the other levorotatory to exactly the same degree.

* Van't Hoff's "La Chimie dans l'Espace" (1875).

† Bull. soc. chim. (1874), p. 337.

**Optical Inactivity of Sugars.** *External Compensation.* — Van't Hoff * called attention to the important fact that when a compound with an asymmetric carbon atom is produced in the vegetable or animal organism it is found in most cases to possess optical activity. When, however, such a compound is formed synthetically, from an inactive substance, optical activity is wanting. Van't Hoff showed that in the latter case inactivity was due to the two opposite isomers being produced in exactly equal amounts, whereas in nature only one of these isomers is formed. Thus the fructose produced in nature is levorotatory; the fructose made synthetically from acrolein dibromide is optically inactive, and consists of equal proportions of left-rotating and right-rotating sugar. If the synthetic fructose be fermented, however, the left-rotating sugar is destroyed, when the unfermented isomer will polarize to the right.

*Internal Compensation.* — In addition to the above case of external compensation between two asymmetric carbon compounds, there is also the case of optical inactivity through internal compensation between two opposite symmetrical halves of the molecule. Thus mesotartaric acid can be given either of the following configurations:




These apparently opposite forms are identical, however, for one configuration can be brought into coincidence with the other by rotating through an angle of 180°. The two **C** atoms printed in heavy type are each asymmetric, yet the compound is inactive, since the optical effect of the one is counterbalanced by that of the other. In such cases of internal compensation the molecule can be divided by a plane of symmetry (indicated above by the dotted line) into two opposite halves, which are mirror images of each other.

Optical inactivity through internal compensation cannot exist with the sugars or their monobasic acids; it is common, however, with the sugar alcohols and dibasic acids. Mesoerythrite, adonite, xylite, dulcite, mucic and allomucic acids, ribo- and xylotrioxyglutaric acids are other examples.

**Nomenclature of Optically Opposite Isomers.** — Since every optically active substance has an antipode, or isomer, of equal but exactly opposite rotation, the nomenclature of such isomers is of considerable

* "Chemistry in Space," Oxford (1891), p. 38.

importance. In only a few cases, as with fucose and rhodeose, where the compounds were named before their antipodal nature was discovered, have wholly distinct names been given to the members of an opposite pair. The optical antipodes of known sugars were first synthesized by Fischer* who adopted the plan of distinguishing such compounds by means of the letters d and l. These symbols, which primarily refer to the character of rotation (*d* = *dexter*, right; *l* = *lævus*, left), were used by Fischer to indicate synthetic relationships rather than directions of rotation. Fischer, starting with the common dextrorotatory sugars, glucose and galactose, gave them the symbols d-, and their opposite isomers the symbols l-. All sugars which could be derived from these sugars synthetically were grouped in the corresponding d- and l- class. Ordinary fructose, or levulose, which though levorotatory can be synthesized from d-glucose, was, therefore, named d-fructose. Ordinary xylose is dextrorotatory but was called l-xylose by Fischer,† because its first discovered synthetic relationship connected it with l-glucose. Salkowski and Neuberg afterwards found that ordinary xylose could be derived from d-glucose through d-glucuronic acid. As Fischer remarks, had this latter relationship been discovered first, he would have named the sugar d-xylose. Such a nomenclature has obviously more historic than scientific value, and various improvements have been proposed by Maquenne,‡ Rosanoff,§ and others. The original system of Fischer, however, is still the one most used and is retained without change in the present volume.

Racemic mixtures, i.e., mixtures of optical antipodes in equal proportions, are necessarily inactive. The combined symbol d, l-, introduced by Fischer, expresses the nature of such a combination more clearly than the symbol i-, which has also been used. The letter i-, however, is sometimes employed to designate iso-, and sometimes to specify a compound which is inactive through internal compensation, the latter use being the one followed in the present work.

**The Formation of Carbohydrates‖ in Nature.** — The carbohydrates are formed primarily only in the plant world, the proximate constituents of their formation being carbon dioxide and water. The combination of these — a process called *assimilation* — is effected only in the green chlorophyll-bearing tissue of the leaves. The carbon dioxide (3 vol-

* Ber., **23,** 370; **40,** 102.

† Ber., **40,** 102.

‡ Maquenne's "Les Sucres."

§ J. Am. Chem. Soc., **28,** 114.

‖ For a very complete treatment of the subject of assimilation and of the origin of carbohydrates in plants the reader is referred to Czapek's "Biochemie der Pflanzen," Jena, 1905, Vol. I, pp. 188–583.

umes of which occur in 10,000 volumes of air) enters the leaf through the breathing pores and there unites with the water which has been drawn up through the roots from the soil. The combination takes place with the liberation of one volume of oxygen for each volume of carbon dioxide assimilated. The process is thus the opposite of respiration and combustion, as is illustrated by the following equations:

*Respiration and Combustion*..... $C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + H_2O.$
Sugar + oxygen = carbon dioxide + water.

*Assimilation*..... $6\,CO_2 + H_2O = C_6H_{12}O_6 + 6\,O_2.$
Carbon dioxide + water = sugar + oxygen.

Assimilation in building up sugar thus plays an important part in purifying the atmosphere and in keeping a balance in the economy of nature.

*Photosynthesis.* Assimilation takes place only by daylight and is most active in the bright sunshine. The chlorophyll grains constitute the mechanism by which the energy of the light waves is transformed into chemical work;* it has been observed that light in passing through the green coloring matter of chlorophyll is changed from shorter into longer wave length, and this phenomenon plays, no doubt, an important part in the process of assimilation.

The many intermediate steps in the process of assimilation are still hidden in obscurity. The most widely accepted view, that of Baeyer,† is that formaldehyde is the first product formed,

$$CO_2 + H_2O = CH_2O + O_2.$$

The fact that formaldehyde is found in green leaves only in the smallest traces is explained by assuming that it immediately undergoes a condensation to form a hexose carbohydrate,

$$6\,CH_2O = C_6H_{12}O_6.$$

The condensation by Loew of formaldehyde to a mixture of hexose sugars has been advanced as an argument in support of this theory.

Opinions differ widely as to the nature of the carbohydrate which is first formed in assimilation. Many plant-physiologists and chemists consider the first product to be glucose, from which all the other carbohydrates are afterwards derived. Others believe starch to be the

* The fact that the light from the sky is more or less polarized has given rise to the hypothesis that the energy of such polarized sunlight produces the optical activity of the sugars which are formed by assimilation. The hypothesis has found no scientific support.

† Ber., **3**, 63 (1870).

first carbohydrate formed and others sucrose. Glucose, fructose, sucrose, maltose, and starch have all been detected in the leaves of plants, but the ease with which the different sugars in nature pass into one another by condensation or hydrolysis makes it difficult to say whether this or that sugar is of primary or secondary origin.

It is well established that the starch of the leaf is one of the products of photosynthesis. If the leaves of plants gathered by daylight be extracted with alcohol to remove the chlorophyll, a distinct blue coloration is produced upon dipping them in iodine solution. This reaction for starch is not obtained, however, with leaves which are plucked before daylight; which proves that light energy is necessary for the formation of starch in the leaf and that the starch which is thus formed is afterwards hydrolyzed into sugar.

*Transportation and Metabolism of Sugars in Plants.* — The sugar, which is produced in the leaf is afterwards transported to various parts of the plant, where it is either transformed into cellulose, hemicellulose, and other substances of the mechanical tissue, or else stored up as reserve material in the form of sucrose, starch, inulin, and other carbohydrates.

The intensity of assimilation has been measured for many different plants. The results are usually expressed in grams of starch or sugar which are formed per square meter of leaf surface in an hour. The determinations show differences for different plants and for different conditions of temperature and sunlight, the results varying from traces up to two grams or more of carbohydrates per square meter of leaf area per hour. Measurements of sunshine, temperature, and leaf area are used in fact as a means of forecasting the probable production of sugar by a beet crop.

# CHAPTER XIX

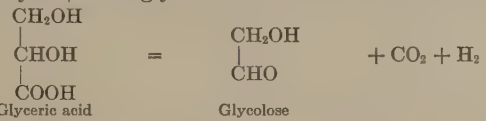
## THE MONOSACCHARIDES

### Dioses

$C_2H_4O_2$

**Glycolose.** — Glycolaldehyde.

Glycolose has not been found as yet free in nature. It has been prepared synthetically by oxidation * of its alcohol glycol with nitric acid and by electrolysis † from glyceric acid.




Glycolose is also obtained by the condensation of two molecules of formaldehyde and in many other ways.

*Properties.* — Glycolose crystallizes in colorless plates, melting at 95° to 97° C., is easily soluble in water and alcohol and has a sweet taste. It is optically inactive and unfermentable. It yields upon oxidation first monobasic glycollic acid, and then dibasic oxalic acid.

*Tests.* — Glycolose gives all the ordinary sugar reactions. $\alpha$-Naphthol and sulphuric acid give a bluish violet coloration ‡ with total absorption of the red and violet parts of the spectrum and a band between the *D* and *E* lines. It forms a number of osazones of which the p-nitrophenyl-osazone is especially characteristic; the compound is very insoluble in the ordinary solvents but can be crystallized from pyridine; its melting point is 311° C.

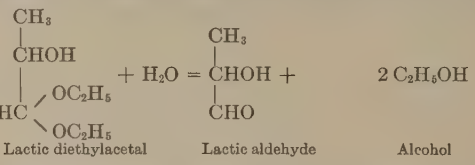
### Methyldioses

$CH_3 \cdot C_2H_3O_2$

**Methylglycolose.** — Lactic aldehyde.

* Fischer and Tafel., Ber., **20,** 1091; **22,** 96. † Neuberg, Biochem. Zeitschr., **7,** 527.
‡ Neuberg, Z. Ver. Deut. Zuckerind., **51,** 271.

This, the simplest of methyl sugars, was prepared by Wohl and Lange* by saponifying its acetal derivative with dilute sulphuric acid.

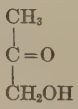



The sugar as thus split off is obtained in a polymerized bimolecular form $(C_3H_6O_2)_2$ consisting of large needles melting at 101° C.; upon heating this polymerized compound the simple monomolecular sugar is obtained.

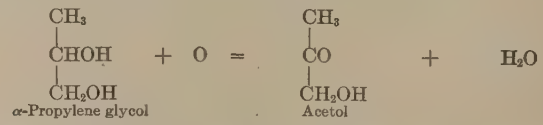
*Properties and Tests.* — Methylglycolose consists of a colorless liquid with slightly rancid odor. It is colored brown by alkalies, reduces Fehling's solution, and gives the other reactions of a simple reducing sugar. Its phenylhydrazone forms colorless leaflets melting at 92° C.; its nitrophenylhydrazone consists of bright yellow prisms melting at 129° C. Its osazone is identical with that of acetol and methylglyoxal.

While methylglycolose has not thus far been found free in nature its monobasic acid derivative lactic acid, $CH_3CHOHCOOH$, is very widely distributed.

**Acetol.** — Acetylcarbinol.




This, the simplest of ketose sugars, can be prepared in a number of ways. It is formed by oxidizing $\alpha$-propylene glycol with bromine water, or by the action of *Bacterium xylinum*.†




Acetol is also formed in large amounts by distilling glucose with very concentrated potassium hydroxide solution.

*Properties.* — Acetol consists of a colorless, sweet-smelling liquid with a nutty flavor, which boils in vacuum at 105 °C. and in air at

* Ber., **41,** 3612.

† Kling, Compt. rend., **128,** 244; **129,** 219, 1252; **133,** 231.

147° C. with decomposition. It is easily soluble in water, alcohol and ether and reduces Fehling's solution strongly in the cold.

*Reactions.* — Acetol gives the oxime, hydrazone, osazone and other reactions common to reducing sugars. The phenylosazone, $C_{15}H_{16}N_4$, is formed by heating acetol with phenylhydrazine and consists of yellow needles melting between 145° and 148° C.; the compound is identical with the osazone of lactic aldehyde and methylglyoxal ($CH_3CO \cdot COH$). Acetol-phenylosazone is also formed * upon heating glucose with phenylhydrazine in alkaline solution, the acetol being first formed as a decomposition product of the glucose and then reacting with the phenylhydrazine.

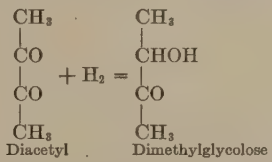
## Dimethyldioses

$(CH_3)_2C_2H_2O_2$

**Dimethylglycolose.** — Dimethylketol. Acetylmethylcarbinol.

*Occurrence.* — Dimethylglycolose is formed in small amounts in many aërobic fermentations of sugars. It is a common constituent of cider vinegar † and is a frequent by-product in the acetic fermentation.

*Synthesis.* — Dimethylglycolose was prepared synthetically by Pechmann ‡ by reducing diacetyl with zinc and sulphuric acid.




*Properties.* — Dimethylglycolose is a colorless liquid boiling at 141° to 142° C., and is easily soluble in water and alcohol. Similar to other sugars of the diose group it is easily polymerized.

*Tests.* — Dimethylglycolose reduces Fehling's solution even in the cold. It is best recognized by means of its yellow finely crystalline phenylosazone, $C_{16}H_{18}N_4$, which is very insoluble in water, alcohol, and ether; the compound is also distinguished by its high melting point,

* Pinkus, Ber., **31,** 31.
† Browne, J. Am. Chem. Soc., **25,** 31.
‡ Ber., **21,** 2754; **22,** 2214; **23,** 2421.

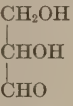
245° C., which seems to be the highest of any phenylosazone thus far prepared.

## Trioses

$C_3H_6O_4$

### aldotrioses

**d, l-Glycerose.** — Glyceric aldehyde.



Glycerose has not been found free in nature, but has been prepared synthetically by oxidation * of its alcohol glycerol, by action of water upon acrolein dibromide, and in other ways.

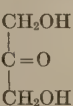
*Properties.* — d,l-Glycerose crystallizes from methyl alcohol in the form of colorless needles melting at 138° C. The compound shows a great tendency to polymerize. It is optically inactive. The fermentation of glycerose sirup by yeast, observed by Fischer and Tafel, is probably due to the formation of a fermentable condensation product. Pure glycerose according to Wohl † and Emmerling ‡ is not fermentable.

*Tests.* — Glycerose reduces Fehling's solution and exhibits all the other reactions common to sugars. Heating with concentrated hydrochloric acid and a little orcin produces a bluish green color § which soon separates as a flocculent precipitate; solution of the latter in amyl alcohol gives a characteristic absorption band between the *C* and *D* lines of the spectrum. Phloroglucin ‖ in presence of a little sulphuric acid gives a flocculent precipitate with dilute glycerose solutions upon warming.

d, l-Glycerose gives glycerol upon reduction, and upon oxidation first monobasic d, l-glyceric acid and then dibasic tartronic acid. The sugar has not been resolved as yet into d- and l-glycerose; although d, l-glyceric acid has been separated by Frankland and Frew ¶ by fermenting calcium d, l-glycerate with *Bacillus ethaceticus* which attacks only the l-component.

### ketotrioses

**Dioxyacetone.** —



* Fischer and Tafel, Ber., **20,** 3384.
† Ber., **31,** 1796, 2394.
‡ Ber., **32,** 544.
§ Neuberg, Z. Ver. Deut. Zuckerind., **51,** 271.
‖ Wohl and Neuberg, Ber., **33,** 3095.
¶ J. Chem. Soc., **59,** 96; **63,** 296.

Dioxyacetone is formed as a by-product in a number of different fermentations. It has been prepared synthetically in several ways, but the best method is that of Bertrand* which consists in fermenting a 5 or 6 per cent glycerol solution with *Bacterium xylinum*. When the reducing power of the solution has reached its maximum, fermentation is interrupted; the solution is evaporated in vacuum, the sirup extracted with 5 to 6 parts alcohol and 2 parts ether, and the dioxyacetone crystallized from the alcohol-ether extract.

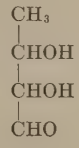
*Properties.* — Dioxyacetone is a white crystalline compound soluble in cold water and boiling alcohol. It has a sweet taste and melts between 68° and 75° C. under polymerization. Its concentrated water solutions also polymerize readily yielding a crystalline compound of melting point 155° C. It is optically inactive and not fermented by yeast.

*Tests.* — Dioxyacetone reduces Fehling's solution even in the cold. Similar to all ketoses it gives the characteristic reaction with resorcin† and an osazone with methylphenylhydrazine. This osazone‡ has the formula $C_{17}H_{20}N_4O$ and melts at 127° to 130° C. Distillation of dioxyacetone with 20 per cent sulphuric acid gives methylglyoxal,§ $CH_3 \cdot COCHO$. Reduction with sodium amalgam gives glycerol‖ quantitatively. Dioxyacetone does not give the reaction with phloroglucin characteristic of the isomeric glycerose.

## Methyltrioses

$CH_3 \cdot C_3H_5O_3$

**Methylglycerose.** —

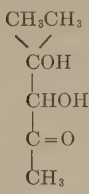


This compound has been prepared synthetically by Wohl¶ and Frank from crotonaldehyde. It forms a colorless sirup easily soluble in water and alcohol and reduces Fehling's solution about half as strong as glucose.

* Compt. rend., **126,** 842, 984.
† Neuberg, Z. Ver. Deut. Zuckerind., **51,** 271.
‡ Neuberg, Ber., **35,** 964.
§ Pinkus, Ber., **31,** 31.
‖ Piloty, Ber., **30,** 1656, 3161.
¶ Ber., **35,** 1904.

### Trimethyltrioses
$(CH_3)_3C_3H_3O_3$

**Trimethyltriose.** —

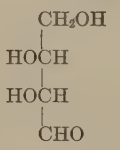


This compound, which belongs to the ketoses, has been made synthetically by Harries and Pappos * from mesityl oxide. It consists of a bright yellow sirup of caramel-like odor, easily soluble in water, alcohol and ether.

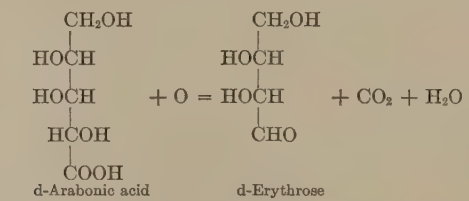
### Tetroses
$C_4H_8O_4$

#### Aldotetroses

**d-Erythrose.** —



This sugar has been prepared synthetically from d-arabonic acid by Wohl † through decomposition of the nitrile with ammoniacal silver solution. Ruff ‡ has also prepared the sugar by oxidation of calcium d-arabonate with hydrogen peroxide in presence of ferric acetate. In this reaction the COOH group of the acid is split off with evolution of $CO_2$.




d-Arabonic acid d-Erythrose

The configuration of d-erythrose is established by means of these reactions.

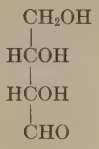
*Properties.* — d-Erythrose consists of a colorless sirup which solidifies to a white mass when dried over phosphorus pentoxide. It is

* Ber., **34**, 2979. † Ber., **26**, 743. ‡ Ber., **32**, 3672.

easily soluble in water and alcohol. The sugar is optically active and exhibits mutarotation; $[\alpha]_D = -14.5$ ($+1.0$ in fresh aqueous solution). d-Erythrose is not fermented by yeast.

*Tests.* — d-Erythrose reduces Fehling's solution and gives all other reactions common to reducing sugars. Reduction with sodium amalgam gives optically inactive mesoerythrite, which is widely distributed in nature in different algæ and lichens. Oxidation of d-erythrose produces first monobasic d-erythronic acid and then dibasic mesotartaric acid.

**l-Erythrose.** —

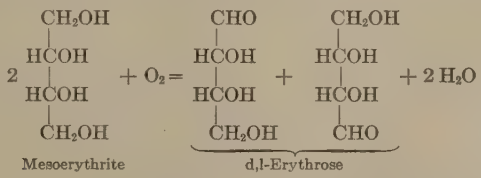


This sugar has been prepared synthetically from l-arabonic acid according to the methods of Wohl* and Ruff† described under d-erythrose. Neuberg‡ has also prepared the sugar from l-arabonic acid by his method of electrolysis.

*Properties.* — l-Erythrose consists of a colorless sweet sirup which has not as yet been obtained crystalline. The sugar is dextrorotatory, $[\alpha]_D = +32.7$ (Wohl); Ruff and Meusser§ found $[\alpha]_D = +21.5$ constant and in fresh solution $+2.4$. The differences noted are probably due to the fact that the sugar has not yet been isolated in the pure condition. l-Erythrose is not fermentable.

*Tests.*—l-Erythrose gives all the ordinary reactions of reducing sugars. Reduction with sodium amalgam gives inactive mesoerythrite the same as d-erythrose; oxidation produces first monobasic l-erythronic acid and then dibasic mesotartaric acid.

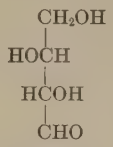
**d, l-Erythrose.** — Racemic erythrose is formed by the oxidation‖ of natural mesoerythrite.



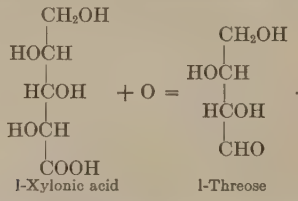

* Ber., **32**, 3666. † Ber., **34**, 1366. ‡ Biochem. Zeitschr., **7**, 527.
§ Ber., **34**, 1366. ‖ Fischer and Tafel, Ber., **20**, 1090.

The sugar is of course inactive. Oxidation produces first d, l-erythronic acid and then mesotartaric acid.

**l-Threose.** —



This tetrose sugar has been formed synthetically by oxidation * of calcium l-xylonate with hydrogen peroxide and ferric acetate.




l-Xylonic acid l-Threose

The configuration of l-threose is established by this reaction.

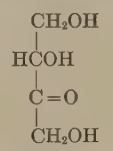
*Properties.* — l-Threose has only been obtained in a sirupy condition, all attempts to promote crystallization having failed.

*Tests.* — l-Threose upon reduction gives l-erythrite ($[\alpha]_D$ in water = +4.25). Oxidation gives first l-threonic acid and then l-tartaric acid.

**d-Threose.** — The optical antipode of l-threose has not as yet been prepared. Its alcohol d-erythrite, however, has been obtained ($[\alpha]_D$ in water = −4.40) by reduction of d-erythrulose.

KETOTETROSES

**d-Erythrulose.** —



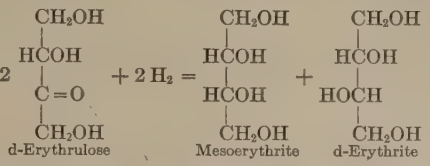
This sugar is best prepared by oxidation of natural mesoerythrite by means of *Bacterium xylinum* according to Bertrand's † method.

*Properties.* — d-Erythrulose has been obtained only as a sirup; it is very soluble in water and alcohol, and is dextrorotatory, the rotation increasing after solution. The sugar is unfermentable.

*Tests.* — d-Erythrulose gives the ordinary ketose reactions, produc-

* Ruff and Kohn, Ber., **34,** 1370. † Compt. rend., **130,** 1330.

ing a coloration with resorcin and hydrochloric acid and resisting oxidation with bromine water. Reduction with sodium amalgam gives both meso- and d-erythrite.




This property of yielding two different alcohols upon reduction is a characteristic of the ketose sugars.

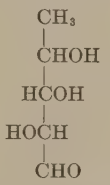
**d, l-Erythrulose** is formed according to Neuberg* during the oxidation of mesoerythrite by hydrogen peroxide in presence of ferrous sulphate (Fenton's† synthesis).

The sugar has been obtained only as a sirup and has not been resolved as yet into its d- and l- components.

### Methyltetroses

$CH_3 \cdot C_4H_7O_4$

**Methyltetrose.** —




This sugar has been prepared synthetically by Fischer‡ from rhamnonic-acid nitrile by Wohl's method.

*Properties.* — Methyltetrose has not been obtained in a pure crystalline form, but only as a yellowish sweet sirup easily soluble in water and alcohol with levorotation, $[\alpha]_D = -5.1°$ (in water).

*Tests.* — Methyltetrose gives the ordinary reactions of an aldose sugar. Reduction with sodium amalgam gives methylerythrite. Oxidation with bromine gives methyltetronic acid, whose lactone gives $[\alpha]_D = -47.5$. Oxidation with nitric acid splits off the $CH_3$ group with formation of d-tartaric acid.

### Dimethyltetroses

$(CH_3)_2C_4H_6O_4$

**Digitoxose.** — This sugar which has the composition of a dimethyl tetrose, $C_6H_{12}O_4$, has been obtained by Kiliani§ by hydrolysis of digi-

* Ber., **35,** 2627.
† J. Chem. Soc., **71,** 375 (1897).
‡ Ber., **29,** 1377.
§ Ber., **31,** 2454; **34,** 3561.

toxin, a glucoside found in different plants of the *digitalis* family. The formation of digitoxose is supposed to proceed as follows:

$$\underset{\text{Digitoxin}}{C_{34}H_{54}O_{11}} + H_2O = \underset{\text{Digitoxigenin}}{C_{22}H_{32}O_4} + 2\,\underset{\text{Digitoxose}}{C_6H_{12}O_4}.$$

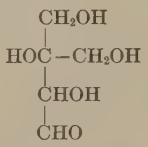
Digitoxose has been obtained as prismatic crystals melting at 101° C., soluble in water and alcohol, and having a specific rotation of $[\alpha]_D = +\ 46°$.

*Tests.* — Oxidation with silver oxide gives among other products considerable acetic acid. Heated with concentrated sulphuric acid and 1 per cent ferrous sulphate, digitoxose solutions are colored, after 30 minutes, a deep blue, which changes in an hour or two to bluish green.

### Oxymethyltetroses

$CH_2OH \cdot C_4H_7O_4$

**Apiose** — β-Oxymethyltetrose.

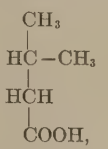


This sugar, which has the same empirical formula $C_5H_{10}O_5$ as a pentose, has been found by Vongerichten* as a constituent of the glucoside, apiin, which occurs in the parsley plant. Apiin upon treatment with strong acids is hydrolyzed as follows:

$$\underset{\text{Apiin}}{C_{26}H_{28}O_{14}} + 2\,H_2O = \underset{\text{Apiose}}{C_5H_{10}O_5} + \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Apigenin.}}{C_{15}H_{10}O_5}.$$

Apiose has been obtained only as an optically inactive, unfermentable sirup.

*Tests.* — Apiose is distinguished from the pentose sugars by not giving furfural upon heating with hydrochloric acid. Reduction with hydriodic acid and phosphorus gives isovaleric acid,



which confirms the branched structure of the carbon chain assigned to apiose.

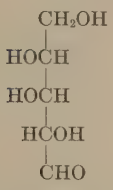
* Ann., **318,** 121; **321,** 71.

PENTOSES

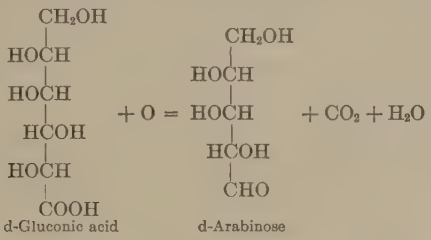
$C_5H_{10}O_5$

ALDOPENTOSES

**d-Arabinose.** —



This sugar, which has been found in nature only as a constituent of d,l-arabinose in abnormal urines, has been prepared synthetically by Wohl's* method from the nitrile of d-gluconic acid and by Ruff's† method from the calcium salt of d-gluconic acid. The oxidation of d-gluconic acid to d-arabinose proceeds as follows:




The configuration of d-arabinose is established by means of this reaction.

*Properties.* — d-Arabinose consists of beautiful prismatic needles melting at 160° C. and easily soluble in water, but insoluble in absolute alcohol. The sugar shows in aqueous solution ($c = 9.45$) $[\alpha]_D = -105°$ (constant); mutarotation is present; d-arabinose is not fermentable.

*Tests.* — d-Arabinose reduces Fehling's solution, yields furfural upon distillation with hydrochloric acid and gives the other reactions characteristic of an aldopentose sugar. Reduction with sodium amalgam gives d-arabite, $C_5H_{12}O_5$, for which $[\alpha]_D = +7.7$ (in saturated borax solution). Oxidation with bromine gives d-arabonic acid, whose lactone $C_5H_8O_5$ gives $[\alpha]_D = +73.73$. Oxidation with strong nitric acid gives d-trioxyglutaric acid, $[\alpha]_D = +22.8$. Especially characteristic of d-arabinose is the very difficultly soluble l-menthylhydrazone‡ which separates in

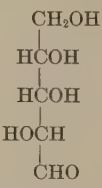
* Ber., **26,** 730.

† Ber., **31,** 1573; **32,** 550; **33,** 1799; **35,** 2360.

‡ Neuberg, Ber., **36,** 1194.

colorless crystals melting at 131° C. and from which d-arabinose can be isolated by decomposition with formaldehyde.

L-ARABINOSE. —



**Occurrence.** — Ordinary or l-arabinose has not been found free in nature except as a constituent of d, l-arabinose in abnormal urines; parent substances, from which l-arabinose may be derived by hydrolysis, are, however, very widely distributed in nature. Chief of these parent substances is the pentosan araban $(C_5H_8O_4)_n$ which occurs as a constituent of many plant gums (cherry gum, peach gum, gum arabic, gum tragacanth, etc.), of the hemicellulose tissues of vegetable cells (sugar beet, maize stalks, elder pith, sugar cane, bran, etc.), and of many plant mucilages (such as quince) and pectins. l-Arabinose has also been found in several glucosides.

**The Arabans.** — Araban itself $(C_5H_8O_4)_n$ occurs in nature not so much in the free condition as in a combined or associated form. The chemistry of this group of substances is exceedingly complex and a satisfactory classification is impossible. Among the arabans, or substances which yield l-arabinose upon hydrolysis, are metaraban, glucoaraban, galactoaraban, arabinic acid, pectose, pectin, parapectin, metapectin, parapectic and metapectic acids, and many other ill-defined substances. The early investigators in this field were hampered by a lack of satisfactory methods and many of the substances, to which they gave separate names, would, if purified, no doubt prove to be identical.

A comparatively pure araban has been prepared by digesting sugar-beet pulp,* and other hemicelluloses,† with dilute alkalies. The clear filtrate is precipitated with weak acids in presence of alcohol. The precipitate, after washing with strong alcohol, is purified by dissolving in water, and reprecipitating with alcohol. The product, after drying, consists of a white amorphous mass, soluble in water to a neutral solution, does not reduce Fehling's solution and is strongly levorotatory ($[\alpha]_D$ given by different authorities varies from $-84$ to $-123$; these

* Ullik, Oest. Ungar. Z. Zuckerind., **23**, 268.

† Schulze, Z. physiol. Chem., **16**, 386.

variations are probably due to differences in the purity of the product). Upon heating with 1 per cent sulphuric acid araban is quickly hydrolyzed to l-arabinose.

$$\underset{\text{Araban}}{(C_5H_8O_4)_n} + n\,H_2O = n\,\underset{\text{l-Arabinose}}{C_5H_{10}O_5}.$$

*Metaraban** is found in the bran of rye, wheat and other cereal grains. The bran, after removing the starch, is heated 3 hours with 1 per cent ammonia, and then filtered and washed with water. The residue is then cooked under pressure with dilute sodium hydroxide which dissolves the metaraban. The latter is precipitated from the filtered solution by means of dilute hydrochloric acid and alcohol. The precipitate, after washing with alcohol and drying, forms a white amorphous substance, which swells up in water and finally gives a mucilaginous, slightly levorotatory solution. Hydrolysis with acids gives l-arabinose.

*Arabinic Acid*† (arabin, metapectic acid) occurs in combination with potassium, calcium and magnesium as the principal constituent of gum arabic and the gum of the cherry, peach, plum and many other trees. It is also produced by the action of alkalies upon pectose and other pectin substances. Arabinic acid can be prepared by dissolving gum arabic‡ in 10 parts of water, acidifying with acetic acid to break up mineral combinations and then dialyzing, or washing, in acetic-acid solution to remove soluble salts and other impurities. The product is purified by dissolving in water and precipitating with alcohol; it is then dried over sulphuric acid at a low temperature, preferably in a vacuum.

Arabinic acid can also be prepared, but in a less pure condition, by the action of alkalies upon beet pulp.§ The latter, after extraction with water and cold 85 per cent alcohol, is boiled in water until all alcohol is expelled and then cooked with an excess of caustic lime. The solution of lime arabinate is filtered and the lime precipitated by means of carbon dioxide, or oxalic acid. The solution is again filtered and the arabinic acid precipitated by adding an excess of strong alcohol. The crude acid is purified and dried as previously described.

Arabinic acid is a white vitreous amorphous substance. Before being dried it is easily soluble in water to an acid solution; but the dry product swells up with water to a mass of almost neutral reaction — a change which is attributed to a conversion of the acid into its lactone.

* Steiger and Schulze, Ber., **23**, 3110.

† Neubauer, J. prakt. chem., **62**, 193 (1854), Scheibler, Ber., **1**, 58; **6**, 612.

‡ O'Sullivan, J. Chem. Soc., **45**, I, 41, Proc. Chem. Soc., **17**, 156.

§ Scheibler, Z. Ver. Deut. Zuckerind., **23**, 288.

The $[\alpha]_D$ of arabinic acid of different origins varies from over $-80$ to over $+80$. Distillation with hydrochloric acid gives large amounts of furfural and oxidation with nitric acid considerable mucic acid. Herzfeld* obtained from a levorotatory arabinic acid 15.3 per cent furfural and 11.5 per cent mucic acid and from a dextrorotatory arabinic acid 5.9 per cent furfural and 41.7 per cent mucic acid. It is thus seen that arabinic acid is a galactoaraban of varying composition. Hydrolysis of both levorotatory and dextrorotatory arabinic acid gives a dextrorotatory mixture of l-arabinose and d-galactose. Neubauer assigned the formula $(C_{12}H_{22}O_{11})_n$ to arabinic acid; O'Sullivan has given the formula $C_{91}H_{142}O_{74}$. Such differences necessarily follow from the variable character of the substance.

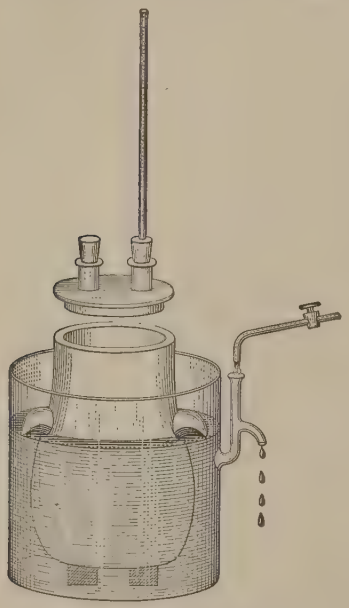


Fig. 190. — Tollens's apparatus for hydrolyzing plant and animal substances.

*Metarabin* is obtained by heating arabinic acid to slightly above 100° C., at which temperature water is given off. It is insoluble in water, yielding only a swollen gelatinous mass. The formula has been given as $(C_{12}H_{20}O_{10})_n$.

Other mixed arabans, as arabogalactan and the pectin substances, are described under d-galactose.

**Preparation of l-Arabinose.** — l-Arabinose can be prepared by the hydrolysis of araban, metaraban or arabinic acid; it is more convenient, however, to prepare the sugar by the direct hydrolysis of certain gums. Cherry gum is one of the purest sources of supply, and as the preparation of arabinose from this substance is typical of other hydrolytic processes, the following method of Tollens† will be described in fuller detail.

*Hydrolysis of Cherry Gum.* — Treat 1000 gms. of pulverized cherry gum in a large porcelain pot with 7000 c.c. of water and 280 gms. of concentrated sulphuric acid, thus making a mixture of about 4 per cent acid. The pot is immersed in a boiling-water bath, as shown in Fig.

* Z. Ver. Deut. Zuckerind., **41**, 667.

† " Handbuch der Biochemischen Arbeitsmethoden " (1902), Vol. II, 65.

190, and the mixture stirred until the gum has dissolved. The pot is then covered and the heating continued for 5 hours. The liquid, which smells strongly of furfural, is then poured into a large evaporating dish, and while still hot neutralized with an excess (300 to 320 gms.) of precipitated calcium carbonate, which must be free from

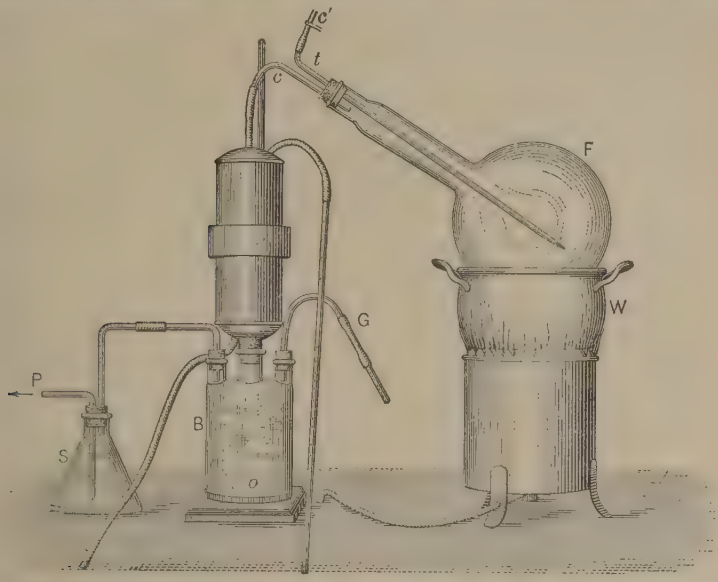



Fig. 191.—Tollens's apparatus for evaporating sugar solutions under reduced pressure.

magnesium carbonate (the magnesium sulphate which is formed interfering with the crystallization of the sugar). The liquid after cooling is filtered through a heavy linen bag, and the precipitate of gypsum, etc., squeezed out in a press (Fig. 131) to remove as much as possible of the liquid. The hydrolyzed solution reduces Fehling's solution strongly, and contains in addition to arabinose more or less galactose and glucose. In order to remove the latter, the solution is poured into a large bottle and fermented in a warm place with a little pure pressed yeast. When fermentation is complete (3 to 4 days at most), the solution is filtered and evaporated under diminished pressure to a sirup.

*Evaporation Under Reduced Pressure.* — In conducting the evaporation of sugar solutions a small laboratory vacuum pan may be used to advantage. In place of such a pan the arrangement of Tollens shown in Fig. 191 may be used to equal advantage. The liquid is placed in

the large balloon flask $F$ of heavy glass, which rests in an inclined position upon the hot-water bath $W$. The flask is closed with a two-hole rubber stopper, which receives through one opening the tube $t$; the latter, drawn out to a fine point, reaches nearly to the bottom of the flask and is fitted at the outer end with a rubber tube and pinch cock $c'$. The flask is connected by the bent tube $c$ to a vertical condenser, which fits into a large Woulf bottle $B$. The latter is connected upon one side with a closed outlet tube $G$ and upon the other side with a safety bottle $S$ to which the suction pump is attached.

In making an evaporation the pump is started and a gentle current of air drawn through the liquid while the bath is being heated. By diminishing the air, the pressure is reduced so that the solution soon begins to boil. The current of air is always maintained slightly so as to keep the liquid in motion and prevent bumping. When it is desired to empty the receiver the pinch cock $c'$ is opened and the distillate siphoned off at the outlet $G$.

*Purification of Sirups.* — When the liquid in the flask has been concentrated to a sirup, the latter is poured out and a fresh quantity of solution evaporated. The concentrated sirups are then united and shaken with 4 to 5 volumes of hot 96 per cent alcohol. After the deposit of gums and mineral matter has settled, the alcoholic solution is filtered and evaporated under reduced pressure to a second sirup. If the latter be very dark in color, it may be further purified by shaking out again with warm alcohol to which a little ether may be added to increase the precipitation of gum. An excess of ether must be avoided as it precipitates part of the sugar. The final sirup, which should be light colored, is set aside in a cool place.

*Crystallization.* — The crystallization of arabinose from sirups prepared from cherry gum is usually rapid; it may be hastened by priming the sirup with a minute crystal of sugar from a stock preparation. When crystallization is complete, the crystals of sugar are sucked off upon a filter, washed with a little alcohol and ether, and air dried. If the sugar is not perfectly white, it may be purified by recrystallizing from hot alcohol after filtering through bone black. The yield of l-arabinose from cherry gum by the above process is about 20 per cent.

**Properties.** — l-Arabinose crystallizes in beautiful prismatic needles melting at 160° C., easily soluble in water but insoluble in absolute alcohol and ether. The sugar shows strong mutarotation; $[\alpha]_D = +104.5$ (constant in aqueous solution).

l-Arabinose is not fermented by yeast; many bacteria, however, are able to set up destructive changes with formation of lactic, acetic,

formic, succinic, oxalic and other acids. *Bacterium xylinum* oxidizes the sugar to l-arabonic acid.

**Tests.** — l-Arabinose gives all the general reactions described for reducing sugars and the furfural, color and other special reactions described for pentoses. The best method for detecting l-arabinose in presence of other sugars (as in hydrolyzed plant materials) is by means of the hydrazone reaction with different substituted hydrazines, such as bromophenylhydrazine,* methylphenylhydrazine,† benzylphenylhydrazine ‡ and diphenylhydrazine.§ The latter reagent is considered to be the best and produces in alcoholic solution in the cold, even with small amounts of l-arabinose, a difficultly soluble hydrazone, $C_{17}H_{20}N_2O_4$, consisting of white needles and melting at 204° to 205° C.‖

The hydrazones of l-arabinose yield, upon decomposition with formaldehyde (p. 348), the free sugar which may then be crystallized and further identified by determining its specific rotation.

Sodium amalgam reduces l-arabinose to l-arabite $C_5H_{12}O_5$, for which $[\alpha]_D = -5.3$¶ (in saturated borax solution), and $-42$ ** (in acid ammonium molybdate). Oxidation of l-arabinose with bromine gives l-arabonic acid, whose crystalline lactone $C_5H_8O_5$ gives $[\alpha]_D = -73.9$. Oxidation of the sugar with strong nitric acid gives l-trioxyglutaric acid, $[\alpha]_D = -22.7$.

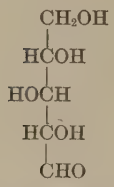
**d, l-Arabinose.** — This sugar, which is a racemic mixture of d- and l-arabinose, has been found as a constituent †† of abnormal urines. The sugar may also be prepared by dissolving equal parts of d- and l-arabinose in hot alcohol and allowing the solution to crystallize.

*Properties.* — d,l-Arabinose forms colorless prismatic crystals of higher melting point (164° C.) and lower solubility than either of its components. The sugar is optically inactive and is not fermented by yeast.

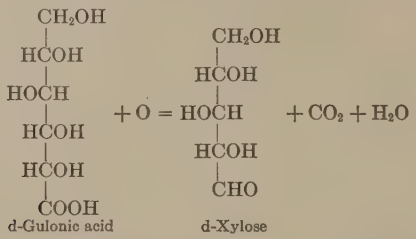
*Tests.* — Reduction of d, l-arabinose with sodium amalgam gives d,l-arabite; oxidation with bromine gives d, l-arabonic acid and with nitric acid d,l-trioxyglutaric acid. Neuberg ‡‡ has resolved the sugar into its components by means of l-menthylhydrazine, which forms a very difficultly soluble hydrazone with d-arabinose, but not with l-arabinose (see pages 362 and 545).

* Fischer, Ber., **24,** 4214; **27,** 2490.
† Ruff and Ollendorff, Ber., **32,** 3234.
‡ Ruff and Ollendorff, Ber., **32,** 3234.
§ Neuberg, Ber., **33,** 2254; **37,** 4616.
‖ Müther and Tollens, Ber., **37,** 312.
¶ Fischer, Ber., **24,** 1836.
** Gernez, Compt. rend., **112,** 1360.
†† Neuberg, Ber., **33,** 2243.
‡‡ Ber., **36,** 1194.

**d-Xylose.** —



This sugar has not thus far been found in nature either in the free or combined form. It has been made synthetically by Fischer and Ruff* from d-gulonic acid by oxidation of the calcium salt with hydrogen peroxide in presence of ferric acetate (Ruff's method).

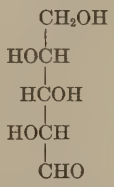



This reaction establishes the configuration of d-xylose.

*Properties.* — d-Xylose crystallizes in white needles melting at 141.5° to 143° C. and giving a constant specific rotation $[\alpha]_D^{20} = -18.6$. In all other respects, except rotation, the sugar resembles its antipode, ordinary l-xylose.

*Tests.* — d-Xylose gives the general reactions of reducing sugars and all the special tests described for pentoses. Oxidation with bromine gives d-xylonic acid whose cadmium double salt $(C_5H_9O_6)_2\,Cd + CdBr_2 + 2\,H_2O$ is especially characteristic. This salt resembles the similar compound of l-xylonic acid which is described under l-xylose.

**L-XYLOSE.** — Wood sugar.



**Occurrence.** — Ordinary, or l-xylose has not been found free in nature except perhaps in rare cases in the urine. The parent substances,

* Ber., **33**, 2142.

from which l-xylose may be derived by hydrolysis, are, however, among the most widely distributed substances in the vegetable world. Chief of these parent substances is the pentosan *xylan* $(C_5H_8O_4)_n$ or *wood gum*, which occurs as a constituent of the incrusting or hemicellular materials which are found in nearly all vegetable cells. Xylan with a little araban makes up from 25 to 30 per cent of the dry matter of cereal straws and grasses, about 15 to 25 per cent of the dry matter of the wood of deciduous trees and from 5 to 15 per cent of the dry matter of the wood of coniferous trees. It is also found in large quantities in bark, roots, bran of seeds and grains, mosses, fungi and associated with araban as a constituent of many vegetable gums. Xylan is, next to cellulose, the most abundant of plant constituents.

**Preparation of Xylan.** — One of the best sources for preparing xylan is beech-wood sawdust, although maize stalks, straw and other plant materials may be used. According to the method of Wheeler and Tollens* 1 kg. of fine beech-wood sawdust is first treated in the cold for 24 hours with 1 to 2 per cent ammonia to dissolve albuminoids, etc.; the ammoniacal solution is then pressed out and the process repeated for a second or third time. The material after washing with water is then treated in a warm place with 5 per cent sodium hydroxide solution, the latter being added in sufficient quantity to form a thick mush. After 24 hours the extract is pressed out and the digestion repeated for another 24 hours using less sodium hydroxide solution. The extracts are mixed and allowed to stand in a flask for deposition of suspended impurities. The clear brown colored solution is then siphoned off and mixed with an equal volume of 96 per cent alcohol which precipitates the xylan as a sodium-gum compound. The latter is filtered off upon cloth, washed with alcohol till the washings are colorless and then treated in presence of alcohol with hydrochloric or acetic acid until the reaction is slightly acid. The free xylan, which is thus liberated, is washed first with alcohol upon cloth, or parchmentized paper, using suction, until all acid is removed, then washed with a little ether, and finally dried over concentrated sulphuric acid. The product thus prepared consists of a grayish white amorphous powder, which is almost insoluble in water. In alkaline solution it is levorotatory, $[\alpha]_D = -70$ to $-90$ according to the purity and origin of the gum. Xylan upon heating with dilute hydrochloric or sulphuric acid is quickly hydrolyzed to l-xylose.

$$\underset{\text{Xylan}}{(C_5H_8O_4)_n} + n\,H_2O = n\,\underset{\text{l-Xylose}}{C_5H_{10}O_5}.$$

* Z. Ver. Deut. Zuckerind., **39,** 848, 863.

*The Xylo-proteids.*—l-Xylose has also been found widely distributed in the animal world as a constituent of many nucleo-proteids. The latter are complex compounds of variable composition and are resolved by hydrolysis into a mixture of substances, among which the nitrogenous bases (adenine, guanine, xanthine and hypoxanthine), phosphoric acid and various sugars have been identified. l-Xylose seems to be the most abundant of the sugars entering into the composition of the nucleo-proteids although other pentose and hexose sugars have been identified. The amount of pentose sugar in different organs has been found by Grund * to be 0.021 per cent in muscle, 0.090 per cent in the brain, 0.081 per cent in the spleen, 0.084 per cent in the kidney, 0.110 per cent in the liver, and 0.447 per cent in the pancreas; it is especially in the pancreas that the occurrence of l-xylose in the animal body is localized. The origin of l-xylose in the animal organism is not absolutely known although Neuberg and Salkowski † regard d-glucuronic acid as the parent substance from which it is derived. The nucleo-proteids are also widely distributed in the vegetable kingdom and give the same products upon hydrolysis.

**Preparation of l-Xylose.**—l-Xylose may be prepared by hydrolysis of xylan obtained as described above or by direct hydrolysis of plant materials. Xylan may be hydrolyzed with sulphuric acid in the same way as described for cherry gum or with hydrochloric acid according to Councler's ‡ method. For the latter process 15 gms. of xylan are heated on the water bath with 200 c.c. water and 70 c.c. hydrochloric acid (1.19 sp. gr.) for 3 hours. The solution is then treated with pure lead carbonate, until Congo-red test paper shows no free acid, and filtered. The filtrate is evaporated to a thin sirup in presence of a little lead carbonate and then treated with strong alcohol to precipitate gums, lead chloride and other impurities. The alcohol solution is treated with hydrogen sulphide to precipitate any remaining lead, filtered and evaporated to a sirup in presence of a little calcium carbonate. The bright straw-colored sirup thus obtained is set aside in a cool place when crystallization of xylose will proceed rapidly. The crystals are purified by recrystallizing from alcohol using a little animal charcoal.

l-Xylose can also be prepared directly from wheat straw, maize stalks, etc., by the process of Schulze and Tollens.§ The material is first purified by digesting with 2 per cent ammonia and water (as described under preparation of xylan), and then, after pressing as dry as

* Z. physiol. Chem., **35,** 111.
† Z. physiol. Chem., **36,** 261; **37,** 464.
‡ Chem. Ztg. (1892), 1719.
§ Ann., **271,** 40.

possible, heated on the water bath 4 hours with 2 to 3 per cent sulphuric acid. The acid extract is pressed out, neutralized with powdered calcium carbonate, filtered and evaporated (as described under preparation of l-arabinose) to a sirup. The latter, after shaking out several times with hot 96 per cent alcohol to precipitate gums, yields upon evaporation 3 to 5 per cent of the weight of straw in crystallized xylose.

**Properties of l-Xylose.** — l-Xylose crystallizes in white needles of a sweetish taste, which are easily soluble in water and hot alcohol, but insoluble in ether. The sugar melts at about 145° C., although different authorities vary 10° C. above or below this figure owing no doubt to variations in method. l-Xylose shows stronger mutarotation than any other sugar; $[\alpha]_D$ 5 minutes after solution $= +\ 85.68$ (Wheeler and Tollens *); $[\alpha]_D$ constant $= +\ 18.5$. The sugar is not fermented by yeast; many bacteria and moulds, however, are able to produce destructive changes with formation of lactic, succinic, acetic and l-xylonic acids.

**Tests.** — l-Xylose gives all the general reactions described for reducing sugars and the furfural and other special reactions described for the pentoses. One of the best methods for detecting xylose in presence of other sugars is *Bertrand's* † *reaction* by means of bromine and cadmium carbonate. The bromine oxidizes the xylose to xylonic acid according to the following reaction:

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_3 \\ | \\ CHO \\ \text{Xylose} \end{array} + H_2O + Br_2 = \begin{array}{l} CH_2OH \\ | \\ (CHOH)_3 \\ | \\ COOH \\ \text{Xylonic acid} \end{array} + \underset{\text{Hydrobromic acid}}{2\,HBr}$$

The xylonic and hydrobromic acid react with the cadmium carbonate forming cadmium xylonate and bromide, the solution of which on evaporation deposits characteristic boat-shaped crystals of the double bromide and xylonate of cadmium $(C_5H_9O_6)_2Cd + CdBr_2 + 2\,H_2O$. The salt can be purified by recrystallizing and should show upon analysis 29.86 per cent Cd and 21.32 per cent Br.

Bertrand's reaction, according to Tollens and Widtsoe,‡ is carried out as follows. For each 0.2 gm. sugar or double the amount of sirup to be tested, 1 c.c. of water, 0.25 gm. bromine (7 to 8 drops) and 0.5 gm. cadmium carbonate are mixed together in a test tube with gentle warming

* Ber., **22,** 1046.
† Bull. soc. chim., [3], **5,** 546.
‡ Ber., **33,** 132.

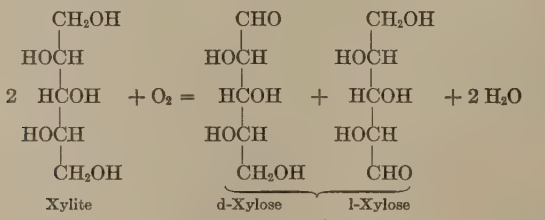
and then, after corking loosely, set aside for 24 hours. The solution is then evaporated in a dish almost to dryness, taken up with a little water, filtered and again evaporated almost to dryness. If xylose is present addition of a little alcohol will soon cause crystals of the double cadmium salt to deposit. Presence of impurities may delay the crystallization somewhat. Too much bromine must be avoided in making the test, and an excess of cadmium carbonate must always be present. The first crop of crystals frequently appear amorphous, but the characteristic boat-shaped needles are always obtained upon recrystallizing.

A second method which has been employed for the detection of l-xylose in impure mixtures is by means of the diformal * compound, which separates in crystalline form upon boiling xylose solutions with paraformaldehyde (trioxymethylene). l-Xylose-diformal has the formula $C_5H_6O_5(CH_2)_2$ and consists of white crystals melting at 56° C.; it can be sublimed without decomposition and shows in methyl alcohol $[\alpha]_D = +25.7$.

l-Xylose upon reduction with sodium amalgam is converted into the optically inactive pentite alcohol, xylite. Oxidation with bromine gives l-xylonic acid and with nitric acid inactive xylotrioxyglutaric acid. l-Xylose has been synthetized from l-gulonic acid by Fischer and Ruff † employing the same method described for d-xylose.

**d, l-Xylose.** — Racemic xylose has been prepared by Fischer and Ruff ‡ by crystallizing a mixture of equal parts of d- and l-xylose out of 96 per cent alcohol. The sugar consists of small prismatic crystals melting at 129° to 131° C. Its phenylosazone melts at 210° to 215° C., whereas the phenylosazone of l-xylose melts at only 160° C.

d, l-Xylose is also formed by the oxidation of inactive xylite by means of bromine.



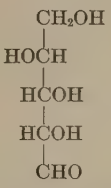

* Lobry de Bruyn and van Ekenstein, Rec. trav. Pays- Bas, **22,** 159.

† Ber., **33,** 2142.

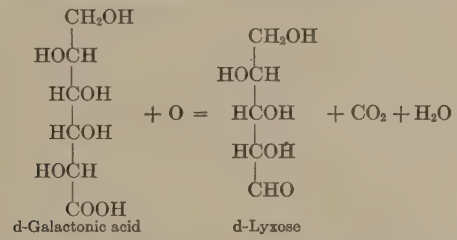
‡ Ber., **33,** 2145.

d-l Xylose has not been resolved as yet into its optically active components.

**d-Lyxose.** —



d-Lyxose has not been identified with certainty in any natural product, although Haiser and Wenzel* believe to have obtained it in the hydrolysis of inosinic acid (a nucleic acid found in meat extract). The sugar has been made synthetically in a variety of ways; by reduction of d-lyxonic lactone, by degradation of d-galactonic nitrile (Wohl's † method) and by oxidation of calcium d-galactonate by hydrogen peroxide in presence of ferric acetate (Ruff's ‡ method).




The configuration of d-lyxose is established by this reaction.

*Properties.* — d-Lyxose consists of large monoclinic crystals melting at 101° C.; the sugar is sweet, strongly hygroscopic and readily soluble in water and alcohol. $[\alpha]_D$ constant = − 13.9: (4 minutes after solution $[\alpha]_D$ = − 3.1). d-Lyxose is not fermented by yeast.

*Tests.* — d-Lyxose gives the general reactions of reducing sugars and the furfural and other special reactions described for the pentoses. Oxidation with bromine gives d-lyxonic acid ($[\alpha]_D$ of lactone = +82.4), which, by heating with pyridine to 135° C., is partially changed to l-xylonic acid (see p. 775). Reduction of d-lyxose with sodium amalgam gives a pentite alcohol which is identical with d-arabite.

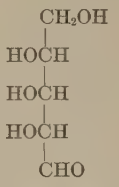
* Monatshefte, **30,** 377; see, however, under d-ribose, the contrary opinion of Levene and Jacobs.

† Ber., **30,** 3105.

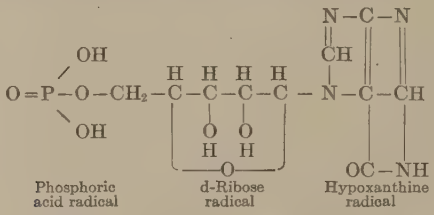
‡ Ber., **32,** 552; **33,** 1798.

l-Lyxose and d, l-lyxose have not as yet been prepared.

**d-Ribose.** —



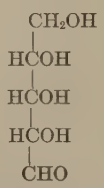
This sugar is regarded by Levene and Jacobs * as a constituent of many nucleic acids in the animal and vegetable kingdom. Inosinic acid according to these authorities has the configuration.




Hydrolysis of the above at nearly neutral point produces free phosphoric acid and the d-ribose-hypoxanthine base; the latter upon further hydrolysis is decomposed into free d-ribose and free hypoxanthine. Levene and Jacobs † have also obtained d-ribose from guanylic acid and yeast nucleic acid.

*Properties.* — d-Ribose as prepared by Levene and Jacobs forms colorless crystals melting at 85° C. and giving $[\alpha]_D^{20} = -19.25°$. The bromophenylhydrazone forms white needles melting at 170° C.

**l-Ribose.** —



This sugar has not been found as yet in any natural product; it has been prepared ‡ synthetically by reducing the lactone of l-ribonic

* J. Am. Chem. Soc., **32,** 231; Ber., **42,** 1198. This view of Levene and Jacobs is contested, however, by Neuberg, Ber., **42,** 2806.

† Ber., **42,** 2474.

‡ Fischer and Piloty, Ber., **24,** 4214.

acid which can be prepared from l-arabonic acid by heating with pyridine (p. 775). From the sirupy mixture obtained by this reduction l-ribose can be precipitated as the phenylhydrazone or bromophenylhydrazone. By decomposition of the latter with benzaldehyde van Ekenstein and Blanksma* were able to isolate the sugar in the pure crystalline form.

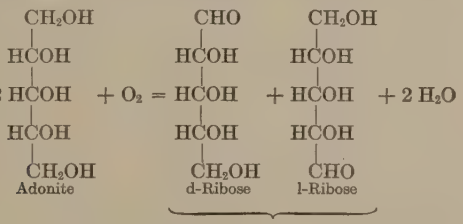
*Properties.* — l-Ribose consists of white needles melting at 87° C., easily soluble in water and alcohol, and showing in aqueous solution $[\alpha]_D = +18.8$ ($c = 1.5$, no mutarotation observable at this dilution).

*Tests.* — l-Ribose gives the ordinary reactions of the pentose sugars. Reduction with sodium amalgam gives the inactive pentite alcohol adonite, which has been found in nature in the juice of the plant *Adonis vernalis.*† Oxidation of l-ribose with bromine gives l-ribonic acid ($[\alpha]_D$ l-ribonic lactone $= -18.0$) and with nitric acid inactive ribotrioxyglutaric acid.

The most characteristic hydrazine derivative is l-ribose-bromophenylhydrazone which consists of colorless crystals melting at 164° to 165° C.

l-Ribose-phenylosazone is identical with that of l-arabinose, as follows from their configuration.

**d, l-Ribose.** — Racemic ribose is formed by the oxidation of natural adonite by means of bromine.




The sugar is also formed by molecular rearrangement from d, l-arabinose by means of dilute alkalies.

d, l-Ribose has not as yet been resolved into its optically active constituents.

**Pentose Sugars of Unknown Character and Constitution.** — In addition to the pentose sugars just described different investigators have reported from time to time a number of other pentose sugars, the

* Chem. Centralbl. (1908), II, 1584; (1909), II, 14.

† Podwyssotzki, Archiv Pharm. (1889), 141.

isolation and identification of which still remain a matter of doubt. Among such pentoses* may be mentioned:

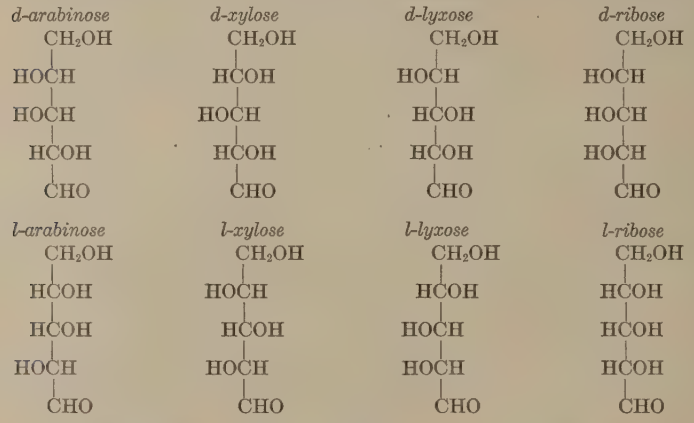
*Cerasinose,* found by Martin in the hydrolytic products of cherry gum.

*Prunose,* found by Garros in the hydrolytic products of plum gum.

*Traganthose,* found by O'Sullivan in the hydrolytic products of gum tragacanth.

*Cyclamose,* found by Michaud in the hydrolytic products of cyclamen bulbs.

It can be shown upon purely theoretical grounds that no other aldopentose sugars can exist than the eight forms already described, viz., d- and l-arabinose, d- and l-xylose, d- and l-lyxose and d- and l-ribose. This may be seen from the following classification:




The possibilities of stereo-isomerism in the aldopentoses are exhausted by the above eight forms, and the existence of new undiscovered sugars in this class is, therefore, precluded. The sugars of unknown character just mentioned either belong to some one of the known pentoses or else fall in another class.

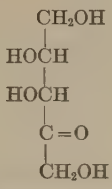
### KETOPENTOSES

None of the ketose sugars belonging to the pentose group has as yet been isolated although several of them have been prepared in an impure form. The number of normal ketopentose sugars theoretically

* See Lippmann's "Chemie der Zuckerarten," p. 154, for a fuller account of these doubtful sugars.

possible is only four; in the ketose class the HCOH group adjoining the aldehyde CHO is replaced by a ketone CO group, while the CHO is itself replaced by a $CH_2OH$ group. It can be seen, therefore, from the preceding classification that d-araboketose is the ketone derivative of d-arabinose and d-ribose; l-araboketose is the ketone derivative of l-arabinose and l-ribose; d-xyloketose is the ketone derivative of d-xylose and l-lyxose; l-xyloketose is the ketone derivative of l-xylose and d-lyxose.

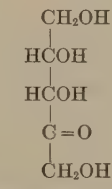
**d-Araboketose.** —



This sugar has been detected together with d-arabinose in the urine of rabbits fed upon d-arabite.* It has also been prepared synthetically by oxidation of d-arabite † with hydrogen peroxide in presence of ferrous sulphate.

*Tests.* — d-Araboketose gives not only the furfural reaction of the pentoses but also the reactions characteristic of the ketoses. It forms an osazone with methylphenylhydrazine, melting at 173° C., thus differing from the aldose d-arabinose. With phenylhydrazine it forms an osazone which is identical with those of d-arabinose and d-ribose (as is necessary from their configuration).

**l-Araboketose.** —

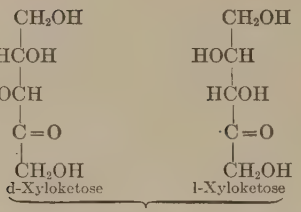


This sugar has been detected by Neuberg ‡ in the oxidation products of l-arabite.

*Tests.* — l-Araboketose gives the furfural reaction of the pentoses and the resorcin and other reactions characteristic of the ketoses. Its phenylosazone is identical with those of l-arabinose and l-ribose (as is necessary from their configuration).

* Neuberg and Wohlgemuth, Ber., **34,** 1745. † Neuberg, Ber., **35,** 962.
‡ Z. physiol. Chem., **31,** 564.

**d, l-Xyloketose.** —

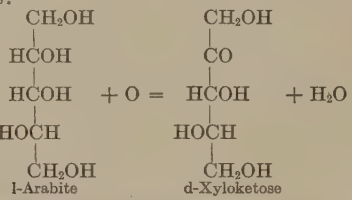



This sugar has been prepared in an impure condition by Neuberg* in the oxidation of xylite by means of lead peroxide ($PbO_2$).

*Tests.* — d, l-Xyloketose gives the ordinary reactions of a ketopentose. Its methylphenylosazone (typical ketose reaction) forms yellow needles melting at 173° C. Its phenylosazone is identical with those of d, l-xylose and d, l-lyxose (as follows from their configuration).

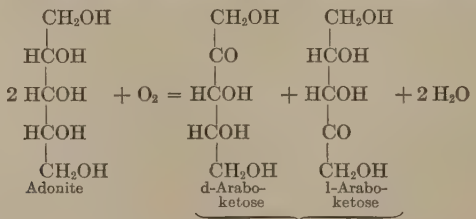
Several ketopentoses are described in the literature under what is apparently a wrong designation.

Bertrand† by the action of *Bacterium xylinum* upon l-arabite obtained a ketose sugar which has been termed l-araboketose. The oxidation of l-arabite by *Bacterium xylinum* must proceed, as is shown on p. 771, as follows:




To produce l-araboketose oxidation would have to take place in a position not open to attack by *Bacterium xylinum.*

Neuberg‡ by oxidation of adonite with lead peroxide obtained a ketopentose which has been designated d,l-riboketose. The oxidation of adonite to form a d, l-ketose must proceed as follows:



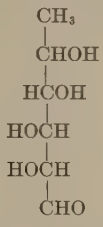

* Ber., **35,** 2628. † Compt. rend., **126,** 762. ‡ Ber., **35,** 2629.

A riboketose must from its configuration necessarily fall in the araboketose class.

### Methylpentoses

$CH_3 \cdot C_5H_9O_5$

**Rhamnose.** — Isodulcite. Rhamnodulcite.



**Occurrence.** — This sugar occurs widely distributed in nature as a component of many vegetable *glucosides*. The latter are condensation products of sugars with alcohols, aldehydes, phenols, acids, oils, resins, alkaloids and other substances; the glucosides are hydrolyzed by acids, and also in most cases by specific enzymes, with liberation of the sugar and other constituents of the glucoside molecule. The following glucosides are named out of a large number which yield rhamnose upon hydrolysis:

*Quercitrin*,* a dyestuff obtained from the bark of *Quercus citrina*. The rhamnose, or isodulcite, sold on the market is nearly all made from this source.

$$\underset{\text{Quercitrin}}{C_{21}H_{22}O_{12}} + H_2O = \underset{\text{Rhamnose hydrate}}{C_6H_{12}O_5 \cdot H_2O} + \underset{\text{Quercetin.}}{C_{15}H_{10}O_7}.$$

*Frangulin*,† a dyestuff from the bark of *Rhamnus frangula*.

$$\underset{\text{Frangulin}}{C_{21}H_{20}O_9} + 2\,H_2O = \underset{\text{Rhamnose hydrate}}{C_6H_{12}O_5 \cdot H_2O} + \underset{\text{Emodin.}}{C_{15}H_{10}O_5}.$$

In many cases a second sugar is associated with rhamnose as a constituent of the glucoside, as

*Sophorin*,‡ a glucoside from Chinese yellow berries.

$$\underset{\text{Sophorin}}{C_{27}H_{30}O_{16}} + 3\,H_2O = \underset{\text{Rhamnose hydrate}}{C_6H_{12}O_5 \cdot H_2O} + \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Sophoretin.}}{C_{15}H_{10}O_7}.$$

*Hesperidin*,§ a glucoside found in many plants of the *Aurantiaceæ*.

$$\underset{\text{Hesperidin}}{C_{50}H_{60}O_{27}} + 3\,H_2O = \underset{\text{Rhamnose hydrate}}{C_6H_{12}O_5 \cdot H_2O} + 2\,\underset{\text{d-Glucose}}{C_6H_{12}O_6} + 2\,\underset{\text{Hesperetin.}}{C_{16}H_{14}O_6}.$$

In the case of glucosides which are hydrolyzed into several sugars, the latter probably exist in the original compound as a higher saccharide;

* Rigaud, Ann., **90**, 283.
† Schwabe, Chem. Ztg., **12**, 229.
‡ Förster, Ber., **15**, 215.
§ Will, Ber., **20**, 1186.

in several cases this higher saccharide has in fact been isolated. Thus *xanthorhamnin*, $C_{34}H_{42}O_{20}$, a glucoside obtained from *Rhamnus sagrada* and other plants, when hydrolyzed by the enzyme *rhamninase*, gives a crystalline trisaccharide sugar rhamninose;* the latter upon heating with dilute acids is hydrolyzed into 2 molecules of rhamnose and 1 molecule of galactose (see page 731).

$$\underset{\text{Rhamninose}}{C_{18}H_{32}O_{14}} + 4\,H_2O = 2\,\underset{\text{Rhamnose hydrate}}{C_6H_{12}O_5 \cdot H_2O} + \underset{\text{d-Galactose}}{C_6H_{12}O_6}.$$

**Preparation.** — The best material for the preparation of rhamnose is the commercial quercitrin or xanthorhamnin. The material is hydrolyzed with dilute sulphuric acid in the same manner as described for l-xylose and l-arabinose. The acid solution is then neutralized by means of barium carbonate and the filtrate evaporated to a sirup under diminished pressure. The sirup thus obtained will deposit crystals of rhamnose hydrate; the yield may be increased by precipitating gums and other impurities from the sirup by means of alcohol. The sugar is purified by recrystallization.

**Properties.** — Rhamnose exists in two forms; as rhamnose hydrate $C_6H_{12}O_5 \cdot H_2O$ and as rhamnose anhydride $C_6H_{12}O_5$.

*Rhamnose Hydrate.* — The common crystalline form of rhamnose consists of large beautiful crystals having a sweetish taste but leaving an after-sensation of bitterness. The melting point of this form of rhamnose is given by different observers from 70° C. to 110° C, a circumstance due to the disturbances produced by the evolution of the water of crystallization. The constant specific rotation of rhamnose hydrate is $[\alpha]_D^{20} = +8.5$; the value decreasing somewhat with increase in temperature (see p. 179). The $[\alpha]_D^{20}$ 2 minutes after solution is $-5°$; this value diminishes and after about 10 minutes $[\alpha]_D = 0$; the rotation then becomes dextrorotatory attaining the constant value $+8.5$ in about one hour.

*Rhamnose Anhydride.* — Rhamnose hydrate begins to give up its water of crystallization at 70° C.; the water is completely removed by drying the sugar in a thin layer at 100° to 105° C., when rhamnose anhydride is obtained as an amorphous vitreous mass. By pulverizing the latter and dissolving it in hot *water-free* acetone, Fischer † obtained the anhydride in the form of white needles melting at 122° to 126° C. The constant specific rotation of rhamnose anhydride is $[\alpha]_D^{20} = +9.4$, which corresponds to the $[\alpha]_D^{20}$ of the hydrate corrected for its water of crystallization. The value for $[\alpha]_D$ of rhamnose anhydride one minute after solution is $+31.5$ (Fischer ‡).

* Tanret, Compt. rend., **129,** 752. † Fischer, Ber., **28,** 1162. ‡ Ber., **29,** 325.

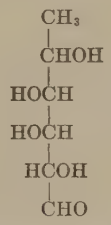
A peculiarity observed in the case of rhamnose is that its alcoholic solution is levorotatory; $[\alpha]_D$ constant in ethyl alcohol $= -9.0$.

Tanret* has explained the peculiar mutarotation of rhamnose hydrate and anhydride by the existence of several isomeric forms.

Rhamnose is not fermented by yeast; certain bacteria, however, bring about destructive changes with production of acetic, lactic and other acids.

**Tests.** — Rhamnose reduces Fehling's solution and gives all the other general reactions characteristic of the reducing sugars. It also gives the group reactions of the methylpentoses, giving methylfurfural upon distillation with hydrochloric acid. Reduction of rhamnose with sodium amalgam produces the methylpentite rhamnite, $C_6H_{14}O_5$, for which $[\alpha]_D = +10.7$. Oxidation of rhamnose with bromine produces rhamnonic acid, whose lactone, $C_6H_{10}O_5$, shows $[\alpha]_D$ of about $-39$.

**FUCOSE.** —



**Occurrence.** — Fucose has not been found free in nature, but its mother substance, a methylpentosan (fucosan), is widely distributed in the vegetable kingdom.

*Fucosan* has been found by Tollens and Widtsoe† in seaweed (varieties of *Fucus*, whence the name fucose), Irish moss, many vegetable gums and other plant materials. It has also been found by Tollens and Oshima‡ in "Nori," a Japanese food product prepared from the purple laver (*Porphyra laciniata*), and seems to be almost universally distributed as a constituent of the marine algæ. Fucosan upon hydrolysis with dilute acids is converted into fucose

$$\underset{\text{Fucosan}}{(CH_3 \cdot C_5H_7O_4)_n} + n\,H_2O = n\,\underset{\text{Fucose}}{CH_3 \cdot C_5H_9O_5}.$$

**Preparation.** — Fucose is best prepared according to the method of Tollens.§ One kilo of dried seaweed (washed as free as possible from sand, etc.) is cut into fine pieces and then treated in the cold for 24 hours with 2 per cent sulphuric acid in order to dissolve mineral salts and other impurities. The seaweed is then pressed, washed several times with cold water and then hydrolyzed with 6 liters of 5 per cent sulphuric

* Compt. rend., **122,** 86. † Ber., **33,** 132. ‡ Ber., **34,** 1422. § Ber., **33,** 132.

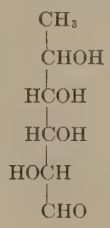
acid for 8 hours in a boiling-water bath. The hot acid extract is then pressed out, neutralized with calcium carbonate and filtered. The filtrate is then evaporated to a sirup in presence of a little calcium carbonate. The sirup is purified by precipitating gums, etc., with alcohol and the alcoholic solution evaporated to a second sirup. The process of purification by means of strong alcohol is again repeated; the final sirup obtained from these purifications is then treated in the cold with phenylhydrazine. After 24 hours the fucose-phenylhydrazone, which has crystallized out, is filtered off, and recrystallized from dilute alcohol. The final product should be nearly white and should melt at about 170° C.

The fucose-hydrazone is then decomposed with benzaldehyde (p. 348) by heating 5 parts hydrazone, 5 parts benzaldehyde, 5 parts alcohol and 4 parts water for one-half to 1 hour upon the water bath in a flask connected with a reflux condenser. After cooling, the solution is filtered from benzaldehyde-hydrazone, shaken out with ether, clarified with animal charcoal and evaporated to a sirup. The latter is set aside in a cool place to crystallize; crystallization can be hastened greatly by priming the sirup with a minute crystal of fucose from a stock preparation. After crystallization is complete the sugar is filtered off (or dried upon an unglazed plate) and recrystallized. The yield of fucose by this process is 3 to 8 grams from 1 kg. of seaweed.

*Properties.* — Fucose consists of microscopic needle-shaped crystals of an agreeable sweet taste, and easily soluble in water. The sugar shows in aqueous solution a constant rotation of about $[\alpha]_D = -75.5$. The rotation immediately after solution exceeds $-124$.

*Tests.* — Fucose gives all the reactions characteristic of the methylpentoses, such as production of methylfurfural upon distillation with hydrochloric acid and the color and spectral reactions described on p. 384. Of its hydrazine derivatives the phenylhydrazone melting at 171° C., the p-bromophenylhydrazone melting at 181° to 183° C., and the diphenylhydrazone melting at 198° C. are among the most characteristic. Oxidation of fucose with bromine gives fuconic acid, $C_6H_{12}O_6$, the lactone of which, $C_6H_{10}O_5$, gives a rotation of $[\alpha]_D = +78.3$.

**RHODEOSE.** —



*Occurrence.* — Rhodeose, the antipode of fucose, has not been found free in nature; it occurs, however, the same as its isomer, rhamnose, as a constituent of certain vegetable glucosides. The best-known glucoside in which rhodeose has been found is convolvulin,* the purgative principle of jalap (*Convolvulus purga*). Convolvulin upon hydrolysis yields convolvulinic acid and a mixture of sugars consisting of glucose, rhodeose and isorhodeose; it is supposed that these sugars are united in the glucoside to form a complex saccharide.

*Preparation.* — Rhodeose is best prepared from the commercial convolvulin; 50 gms. of convolvulin are dissolved in 375 c.c. of barium-hydrate solution (saturated at room temperature). The excess of barium is precipitated by carbon dioxide and sulphuric acid, and the filtrate, which should contain exactly 0.5 per cent free sulphuric acid, heated to 100° C. for 40 hours. The filtered solution is then neutralized with barium carbonate and clarified with 5 c.c. of saturated lead-subacetate solution. The filtrate is freed from lead by means of hydrogen sulphide, and the filtered solution evaporated under reduced pressure to a sirup, which contains the sugars in the proportion of 2 parts of rhodeose to 1 part of glucose. The glucose is removed from the sirup by fermentation with yeast; the rhodeose is then precipitated by means of methylphenylhydrazine as an insoluble hydrazone. The latter after recrystallizing is decomposed by warming several times with benzaldehyde and the filtered solution after shaking out with ether evaporated to a sirup, which is then set aside in a cool place for crystallization. Priming the sirup with a minute crystal of rhodeose from a previous preparation will hasten crystallization.

*Properties.* — Rhodeose consists of small needle-shaped crystals having a sweet taste and easily soluble in water. The sugar shows in aqueous solution a constant rotation of $[\alpha]_D = +75.5$ (+86.5 after solution).

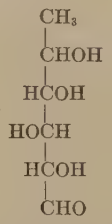
*Tests.* — Rhodeose gives all the reactions characteristic of the methylpentoses. As the optical antipode of fucose it resembles the latter in its behavior with many reagents. The diphenylhydrazone of rhodeose melts at 199° C. (that of fucose at 198° C.); the p-bromophenylhydrazone of rhodeose melts at 184° C. (that of fucose at 183° C.); the methylphenylhydrazone of rhodeose melts at 174° C. (that of fucose at 177° C.), etc. Upon oxidation with bromine rhodeose gives rhodeonic acid, the salts of which have the same composition as those of fuconic acid. The lactone of rhodeonic acid melts at 105.5° C. (that of fuconic acid at 106 to 107° C.); in their rotatory power, however, the

* Taverne, Chem. Centralbl. (1895), I, 56; Votoček, Ber., **37,** 3859; **43,** 469.

two lactones show their antipodal character, $[\alpha]_D$ rhodeonic acid lactone $= -76.3$ (lactone of fuconic acid $= +78.3$).

**Racemic Sugar from Rhodeose and Fucose.** — This racemic combination was prepared by Votoček * by evaporating a solution containing rhodeose and fucose in equal amounts. The sugar was obtained as minute crystals melting at 161° C. and optically inactive; it is much less soluble in water than either of its components.

**Isorhamnose.** —



This methylpentose has not been found in nature; it has been prepared synthetically by reduction of the lactone of isorhamnonic acid (made by heating rhamnonic acid and pyridine to 150° C.).

*Properties.* — Isorhamnose has not been isolated as yet in the crystalline form; as prepared by Fischer and Herborn † the sugar was obtained as a sweet easily soluble sirup which showed a value for $[\alpha]_D$ of about $-30$.

*Tests.* — Isorhamnose gives methylfurfural upon distillation with hydrochloric acid and gives the other reactions of the methylpentoses. Oxidation with bromine gives isorhamnonic acid, the lactone of which shows after solution $[\alpha]_D = -62$, which however sinks in 24 hours to about $-5$, owing to decomposition of the lactone into free acid. The phenylosazone of isorhamnose is identical with that of rhamnose; this of course follows necessarily from the structural relationship of the two sugars.

**Quinovose.** — $CH_3 \cdot C_5H_9O_5$.

*Occurrence.* — This methylpentose has been found as a constituent of the glucoside quinovin, which occurs in the bark of different varieties of the cinchona tree.

*Preparation.* — Quinovin upon hydrolysis with hydrochloric acid in alcoholic solution yields quinovose, which in presence of the alcohol and acid is converted into ethyl quinovoside, $C_6H_{11}O_5 \cdot C_2H_5$. This com-

* Ber., **37**, 3859. † Ber., **29**, 1961.

pound, first called quinovite, was long regarded as a direct product of hydrolysis, until Fischer and Liebermann * ascertained its composition and showed it to be the result of a secondary reaction. The ethyl quinovoside upon heating 1½ hours with 3 parts of 5 per cent sulphuric acid is hydrolyzed into alcohol and quinovose. The solution is diluted with 1 vol. of water, the alcohol evaporated, the acid neutralized with barium carbonate and then, after decolorizing with bone black, the liquid filtered. The filtrate is extracted with ether to remove any unhydrolyzed quinovoside and then evaporated, when the quinovose is obtained as a yellowish sirup.

*Properties.* — Quinovose has been obtained only as a yellowish sirup of strong dextrorotation, easily soluble in water and absolute alcohol.

*Tests.* — Quinovose reduces Fehling's solution, and yields large amounts of methylfurfural upon distillation with 12 per cent hydrochloric acid. Its solutions when heated with phenylhydrazine give quinovose-phenylosazone which consists of fine yellow needles melting at 193° to 194° C. When heated with hydrochloric acid in alcoholic solution quinovose gives the ethylglucoside previously referred to. Ethylquinovoside is an amorphous hygroscopic substance melting at 60° C. and when pure should be perfectly soluble in ether. The compound is dextrorotatory, $[\alpha]_D = +78.1$.

**Isorhodeose.** — $CH_3 \cdot C_5H_9O_5$. This methylpentose of unknown configuration was found by Votoček † together with rhodeose among the hydrolytic products of convolvulin. The sugar has been obtained only as a yellowish sirup of low dextrorotation ($[\alpha]_D = +20$ about). Isorhodeose-phenylosazone consists of fine yellow crystals melting at 190° C.

### SUGARS OF UNKNOWN CONSTITUTION, ISOMERIC OR RELATED TO THE METHYLPENTOSES

**Antiarose.** — $C_6H_{12}O_5$. This sugar was obtained by Kiliani ‡ in the hydrolysis of the glucoside antiarin which is found in the sap of the upas tree (*Antiaris toxicaria*), the milky juice of which is used by Malayans as an arrow poison. Antiarose has only been obtained as a sirup; it gives the reactions of an aldose and, oxidized with bromine, yields antiaronic acid whose lactone, $C_6H_{10}O_5$, is levorotatory, $[\alpha]_D = -30°$.

* Ber., **26,** 2415.
† Ber., **43,** 476.
‡ Archiv. Pharm., **234,** 438.

**Digitalose.** — $C_7H_{14}O_5$. This sugar, discovered by Kiliani,* is supposed to be a dimethylpentose. It is formed by the hydrolysis of the glucoside digitalin, which is found in different species of digitalis.

$$\underset{\text{Digitalin}}{C_{35}H_{56}O_{14}} + 2\,H_2O = \underset{\text{Digitaligenin}}{C_{22}H_{30}O_3} + \underset{\text{Digitalose}}{C_7H_{14}O_5} + \underset{\text{Glucose}}{C_6H_{12}O_6} + 2\,H_2O.$$

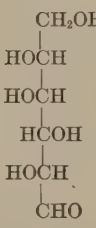
Digitalose has been obtained only as a sirup and gives the reactions of an aldose sugar. Oxidation with bromine gives digitalonic acid $C_7H_{14}O_6$, whose lactone is levorotatory, $[\alpha]_D = -79.4$.

## Hexoses

$C_6H_{12}O_6$.

### ALDOHEXOSES

**D-Glucose.** — Dextrose. Grape sugar. Starch sugar. Diabetic sugar, etc.



**Occurrence.** — d-Glucose is the most widely distributed sugar in nature; it is found in the free condition in the blood and tissues of most animals, and in the juices of nearly all plants. The sugar occurs most abundantly, however, in the combined form in such substances as the vegetable glucosides, the higher sugars and the polysaccharides.

**The Vegetable Glucosides.**† — The reactivity of the glucose molecule in nature is best exemplified by the vegetable glucosides, condensation products of glucose with alcohols, acids, aldehydes, phenols and other compounds. Reference was made to several glucosides which yielded rhamnose upon hydrolysis under the description of the latter sugar. The glucosides, which contain glucose as their sugar constituent, are, however, the most widely distributed in nature. It is impossible to describe all of these, but a few typical examples are given.

* Ber., **25,** 2116; **31,** 2454.

† For a full account of the various glucosides, their preparation, properties, etc., see the section by Euler and Lundberg in the Biochemisches Handlexicon (1911), Vol. II, pp. 578–722; also Armstrong's "Simple Carbohydrates and Glucosides" (1910) and Plimmer's "Chemical Changes and Products resulting from Fermentations" (1903).

The glucosides are usually colorless crystalline compounds with a bitter taste, and for the most part levorotatory.

*Salicin.* — A glucoside found in the bark of the willow and used as a remedy for rheumatism. It is hydrolyzed by the enzyme emulsin to glucose and salicyl (o-oxybenzyl) alcohol.

$$\underset{\text{Salicin}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Salicyl alcohol.}}{C_6H_4(OH)CH_2OH}.$$

*Populin.* — A glucoside found in the bark of several species of poplar (*Populus*). It is hydrolyzed as follows:

$$\underset{\text{Populin}}{C_{20}H_{22}O_8} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Benzoyl-salicyl alcohol.}}{C_6H_5COC_6H_3(OH)CH_2OH}.$$

*Coniferin.* — A glucoside found in the fir and other coniferous trees. It is hydrolyzed as follows:

$$\underset{\text{Coniferin}}{C_{16}H_{22}O_8} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Coniferyl alcohol.}}{C_6H_3\begin{pmatrix}OH\\OCH_3\end{pmatrix}C_3H_4OH}.$$

*Arbutin.* — This glucoside together with *methylarbutin* is found in the leaves of the evergreen bearberry (*Arbutus uva ursi*). The two glucosides are hydrolyzed as follows:

$$\underset{\text{Arbutin}}{C_{12}H_{16}O_7} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Hydroquinone.}}{C_6H_4(OH)_2}.$$

$$\underset{\text{Methylarbutin}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Methylhydroquinone.}}{C_6H_4{\begin{matrix}OCH_3\\OH\end{matrix}}}.$$

*Phloridzin.* — A glucoside found in the bark of the apple, pear and other trees belonging to the Rosaceæ. It possesses the peculiar property of causing glucosuria when taken internally; the amount of glucose in the urine may reach from 6 per cent to as high as 13.5 per cent after ingestion of phloridzin. It is hydrolyzed by acids as follows:

$$\underset{\text{Phloridzin}}{C_{21}H_{24}O_{10}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Phloretin.}}{C_{15}H_{14}O_5}.$$

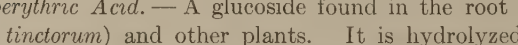
*Gaultherin.* — A glucoside found in the wintergreen. It is hydrolyzed by acids as follows:

$$\underset{\text{Gaultherin}}{C_{14}H_{18}O_8} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Methyl salicylate.}}{C_6H_4OHCOOCH_3}.$$

*Indican.* — A glucoside found in the Indigo plant. It is hydrolyzed by acids or by the enzyme indimulsin as follows:

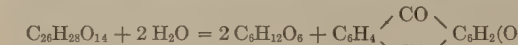
$$\underset{\text{Indican}}{C_{14}H_{17}NO_6} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Indoxyl.}}{C_6H_4{\begin{matrix}NH\\>CH\\COH\end{matrix}}}.$$

The indoxyl of the above reaction is colorless, but undergoes rapid oxidation to indigotin the blue coloring matter of indigo.



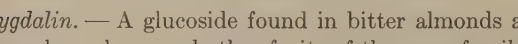
Indoxyl — Indigo-blue.

*Ruberythric Acid.* — A glucoside found in the root of the madder (*Rubia tinctorum*) and other plants. It is hydrolyzed by a specific enzyme or by acids into glucose and the coloring substance alizarin.



Ruberythric acid — Glucose — Alizarin.

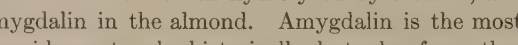
*Amygdalin.* — A glucoside found in bitter almonds and in the kernels of peaches, plums and other fruits of the same family. Amygdalin was the first glucoside to attract investigation; it was discovered in 1830 by Robiquet* and 7 years later Liebig and Wöhler† discovered the manner in which it was hydrolyzed by *emulsin*, an enzyme found with amygdalin in the almond. Amygdalin is the most interesting of the glucosides not only historically but also from the peculiarity of giving off hydrocyanic acid upon hydrolysis.



Amygdalin — Glucose — Benzaldehyde — Hydrocyanic acid.

It has been supposed that the two glucose molecules in amygdalin are united to form a disaccharide. The HCN in amygdalin occurs as the nitrile of l-mandelic acid $C_6H_5CH(OH) \cdot CN$. The monoglucose compound of l-mandelonitrile was obtained by Fischer by hydrolyzing amygdalin with an enzyme found in yeast; it has the following formula, $C_6H_5CH(CN) - O - C_6H_{11}O_5$. This glucoside is also found in nature in the bark of the wild cherry and other trees. There are a large family of glucosides belonging to the mandelonitrile class. Besides amygdalin and its derivative l-mandelonitrile glucoside, the following are mentioned:

*Sambunigrin.* — A glucoside found in the leaves of the common elder (*Sambucus nigra*). It is the monoglucose compound of d-mandelonitrile, the optical antipode of the derivative obtained by Fischer‡ from amygdalin. It is hydrolyzed by acids and emulsin as follows:



Sambunigrin — Glucose — Benzaldehyde — Hydrocyanic acid.

*Prulaurasin.* — A glucoside found in the leaves of the cherry laurel. It is a racemic mixture of d-mandelonitrile glucoside (sam-

* Robiquet and Boutron, Ann. chim. phys. [2], **44**, 352–382 (1830).
† Ann., **22**, 1–24 (1837). ‡ Ber., **28**, 1508.

bunigrin) and l-mandelonitrile glucoside. It is hydrolyzed in the same way as sambunigrin.

*Dhurrin.* — A glucoside found by Dunstan and Henry* in the leaves and stalks of *Sorghum vulgare*. It is a para-hydroxymandelonitrile glucoside and is hydrolyzed by emulsin and acids as follows:

$$\underset{\text{Dhurrin}}{C_{14}H_{17}O_7N} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{p-Oxybenzaldehyde}}{C_6H_4(OH)CHO} + \underset{\text{Hydrocyanic acid.}}{HCN}.$$

The poisoning of cattle by eating sorghum at certain stages of its growth is due to the hydrocyanic acid derived from this glucoside.

*Phaseolunatin.* — A glucoside found by Dunstan and Henry† in Lima beans (*Phaseolus lunatus*), flax and cassava (also termed *linimarin*). It yields the following hydrolytic products:

$$\underset{\text{Phaseolunatin}}{C_{10}H_{17}O_6N} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Acetone}}{CH_3COCH_3} + \underset{\text{Hydrocyanic acid.}}{HCN}.$$

*Sinigrin.* — A glucoside found in the seed of black mustard (*Sinapis* or *Brassica nigra*). It is one of the most interesting of the glucosides, as it contains sulphur and yields a mustard oil as one of its hydrolytic products. The glucoside was first isolated by Bussy who called it potassium myronate. It is hydrolyzed by acids or by the enzyme *myrosin* (which accompanies it in mustard seed) as follows:

$$\underset{\text{Sinigrin}}{C_{10}H_{16}O_9NS_2K} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Allyl mustard oil}}{CH_2{:}CHCH_2\,NCS} + \underset{\substack{\text{Potassium}\\ \text{bisulphate}}}{KHSO_4}.$$

The mustard-oil glucosides constitute a separate class and are peculiar to the plants of the Cruciferæ.

*Sinalbin.* — This is the glucoside of the white mustard (*Sinapis* or *Brassica alba*). It is hydrolyzed by the enzyme *myrosin* which accompanies it in the following manner:

$$\underset{\text{Sinalbin}}{C_{30}H_{42}O_{15}N_2S_2} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\substack{\text{Sinalbin mustard}\\ \text{oil}}}{C_7H_7ONCS} + \underset{\text{Sinapin acid-sulphate.}}{C_{16}H_{24}O_5N \cdot HSO_4}.$$

Glucose is also found associated with tannic and gallic acids as a constituent of another very widely distributed group of plant substances. These have been regarded by some chemists as true glucosides and by others as mere complexes.

*Glucotannin*, a so-called glucoside of this class, is supposed to be hydrolyzed by the enzyme *tannase* as follows:

$$\underset{\text{Glucotannin}}{C_{27}H_{22}O_{17}} + 4\,H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + 3\,\underset{\text{Gallic acid.}}{C_6H_2(OH)_3COOH}.$$

The literature upon the tannin glucosides is in such a contradictory state that no definite compounds can be cited by way of illustration.

* Phil. trans. Roy. Soc., 1902, A **199,** 399.

† Proc. Roy. Soc., **72,** 285.

*Preparation of Glucosides.* — The glucosides are best isolated from plant materials by extraction with alcohol. The extraction should be begun as soon as possible after the material is gathered in order to prevent hydrolysis by accompanying enzymes. In many cases the glucoside will crystallize directly from the alcohol extract; in other cases, where the yield is small, the extract must be concentrated before crystallization will begin. When large amounts of other organic substances are present, clarification with lead salts may be necessary, in which case any excess of lead is afterwards removed by means of hydrogen sulphide.

**Glucose as a Constituent of Higher Sugars.** — Besides forming condensation products with plant alcohols, aldehydes, phenols, acids, etc., to form glucosides, glucose may unite in other ways; it may combine with one or more molecules of itself, or with one or more molecules of other sugars, to form the higher crystalline saccharides. The latter are readily hydrolyzed into the component sugars by acids and specific enzymes in the same manner as the glucosides. The following examples are given of the higher sugars found in nature which yield glucose upon hydrolysis.

| | Sugar | Hydrolytic Products |
|---|---|---|
| Di-saccharides, $C_{12}H_{22}O_{11}$. | Maltose | Glucose + glucose. |
| | Trehalose | Glucose + glucose. |
| | Gentiobiose | Glucose + glucose. |
| | Sucrose | Glucose + fructose. |
| | Turanose | Glucose + glucose. (?) |
| | Lactose | Glucose + galactose. |
| | Melibiose | Glucose + galactose. |
| Tri-saccharides, $C_{18}H_{32}O_{16}$. | Melezitose | Glucose + glucose + glucose. (?) |
| | Gentianose | Glucose + glucose + fructose. |
| | Raffinose | Glucose + fructose + galactose. |
| Tetra-saccharides, $C_{24}H_{42}O_{21}$. | Stachyose | Glucose + fructose + galactose + galactose. |

**Glucose as a Constituent of Polysaccharides.** — In addition to forming higher sugars by condensation of its own molecule, glucose may unite with itself to form the very complex polysaccharides, such as dextrin, dextran, starch and cellulose. The number of molecules of glucose which enter into the structure of these higher derivatives is difficult to determine. Researches by Brown and Morris * indicate that dextrin, the simplest member of the class, contains 40 molecules of glucose in the condensed form and that the molecule of starch is at least 5 times as large as that of dextrin, in other words contains 200

* Chem. Centralbl. (1890), I, 845. See also Brown and Millar, J. Chem. Soc., **75**, 315.

molecules of glucose. Cellulose, the most highly condensed polysaccharide, has without doubt a molecule much greater even than starch. The chemical properties of the higher polysaccharides can only be referred to very briefly.

**Cellulose.** — $(C_6H_{10}O_5)_n$. This is the most abundant constituent in the vegetable kingdom; cellulose in all its various forms probably makes up one-half of the total dry vegetable matter of the world. Cellulose occurs in the pure condition only in the fiber of the cotton ball and in a few similar substances; as a constituent of the cellular tissue of plants cellulose is usually combined with lignin, pentosans and other hemicelluloses to form a complex of varying composition. By treatment of plant membranes with hot solutions of dilute alkalies and acids the lignin, pentosans and other so-called "encrusting substances" are split off from the complex leaving the cellulose behind as an insoluble residue. The latter upon bleaching with chlorine is obtained in a perfectly white fibrous form. The average approximate percentage of cellulose in the water-free substance of different plant materials is as follows:

| Material (water free) | Approximate per cent of Cellulose |
|---|---|
| Wood | 60 |
| Bark | 40 |
| Straw | 40 |
| Leaves | 20 |
| Seeds (including husks) | 15 |
| Roots, tubers, etc. | 10 |

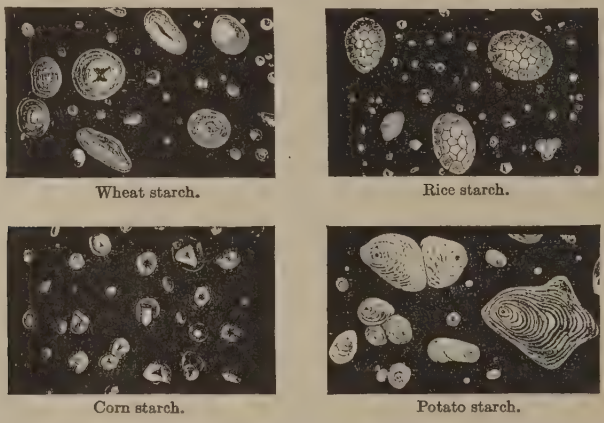
Cellulose, as prepared from its different sources, consists of a white fibrous material insoluble in ordinary solvents but easily soluble in an ammoniacal copper solution. It is reprecipitated from the latter by acids as a gelatinous mass. Cellulose swells up in concentrated sulphuric acid and after short contact is converted into a starch-like substance (amyloid) which is colored blue by iodine. In the same manner cellulose gives a blue coloration with zinc chloride and iodine solution (Schulze's reagent). Cellulose after long contact with concentrated sulphuric acid is dissolved; upon diluting the mixture with water and boiling the cellulose is hydrolyzed to d-glucose.

**Starch.** — $(C_6H_{10}O_5)_{200}$.(?) Starch is next to cellulose the most widely distributed glucose condensation product in the vegetable kingdom. It is stored up as a reserve material in many roots, tubers, grains and seeds, in which parts it may constitute over 90 per cent of the total dry substance. It has been estimated by Nägeli * that 10 per cent of the Phanerogams or flowering plants produce starchy seeds.

* "Die Stärkekörner" (1858), p. 378.

The starch-yielding capacity of certain plants, as the cereals, potato, yam, cassava, etc., is so pronounced as to give them great value in the production of starch for food and industrial purposes. The occurrence of starch in the leaves and chlorophyll tissue of plants has already been referred to.

Starch is deposited in plant cells in the form of minute grains, whose size and shape are peculiar to each botanical species (see Fig. 192). By reducing the starchy parts of the plant to a fine pulp with



Wheat starch. Rice starch. Corn starch. Potato starch.

Fig. 192. — Forms of starch grains. After Möller. (Magnified 400.)

water and straining the milky liquid into a tall cylinder the grains of starch will be deposited as a sediment. The latter is then washed several times by decantation with 0.25 per cent sodium hydroxide solution, which removes protein and other impurities, and then washed with water to remove all traces of alkali. The starch thus prepared is filtered off and dried at a gentle heat. In a similar way, by a process which is largely mechanical, starch is prepared commercially from the potato, cassava, Indian corn, wheat, rice and other plants.

Pure starch, which has not been subjected to heat or strong chemical treatment, occurs in the form of white microscopic granules varying in size from about 0.002 mm. to 0.2 mm. diameter. The granules are insoluble in cold water, but when heated with hot water burst and partially dissolve forming a thick mucilaginous paste.

The conversion of starch by means of diastase and acids is described under maltose.

Starch upon contact with cold hydrochloric acid is converted into

soluble starch. In Lintner's * method the starch is treated for 7 to 8 days with 7.5 per cent hydrochloric acid, after which it is washed free from acid and dried. The product thus obtained dissolves readily in hot water to form a clear limpid solution. The $[\alpha]_D$ of soluble starch ranges from about + 195 to + 200.

**Dextrin.** — $(C_6H_{10}O_5)_{40}$.(?) Dextrin is formed in nature and in the arts by the conversion of starch. It occurs in malted grain and in all starchy seeds during germination. The dextrin molecule is regarded by many chemists as varying in character and the following classification is sometimes made.

(1) Amylodextrin, first dextrin of conversion; blue iodine reaction.
(2) Erythrodextrin, second dextrin of conversion; red iodine reaction.
(3) Achroodextrin, third dextrin of conversion; no iodine reaction.
(4) Maltodextrin, final dextrin of conversion; no iodine reaction.

Numerous subdivisions of dextrins under each of the above groups have also been proposed. The theories concerning the formation of dextrin from starch are discussed under maltose.

A stable dextrin was prepared by Brown and Millar † by precipitating a diastatic starch conversion with alcohol, dissolving the precipitate in water, fermenting away occluded sugars with yeast, and again precipitating with alcohol. After several such purifications and fractional precipitations with alcohol, a dextrin was obtained of only slight reducing power and giving for $[\alpha]_D$ + 195 to + 195.7. The formula $39(C_6H_{10}O_5) \cdot C_6H_{12}O_6$ was assigned to this dextrin (see p. 687).

Dextrin is prepared commercially by heating starch with about 0.2 per cent nitric acid in ovens. The temperature of heating varies according to the color and character of product desired, and ranges from 110° to 170° C. The dextrin thus prepared resembles that obtained from starch by diastatic conversion. It is easily soluble in water, and is precipitated from aqueous solution by strong alcohol. The specific rotation of commercial dextrins will vary according to the method of manufacture (see p. 510). Such dextrins, purified by repeated precipitation with alcohol, will give a value for $[\alpha]_D$ of about +195, the same as that of the stable dextrin prepared by diastase.

Dextrin upon heating with dilute hydrochloric or sulphuric acid is hydrolyzed into d-glucose. The reaction is not perfectly quantitative, however, owing to a slight destruction of the glucose. The rate of hydrolysis was found by W. A. Noyes ‡ and his co-workers to be only about one-half that for maltose.

* J. prakt. Chem., **34** [2], 381. † J. Chem. Soc., **75**, 315.
‡ J. Am. Chem. Soc., **26**, 266.

**Other Glucose-yielding Polysaccharides.** — In addition to the substances previously mentioned glucose occurs in the condensed form in many other plant products, such, for example, as *lichenin** $(C_6H_{10}O_5)_n$, a constituent of many mosses and lichens; *dextran* $(C_6H_{10}O_5)_n$, a mucilaginous substance secreted by many bacteria and resembling dextrin in its properties; *paradextran*† $(C_6H_{10}O_5)_n$, a cellular constituent of many fungi and mushrooms; *plant dextrins* and *gums*, *vegetable-glycogen*,‡ and other materials belonging to the so-called hemicelluloses.

In the animal kingdom glucose is found free in the blood (about 0.1 per cent) and also in very slight amounts in normal urine (usually under 0.01 per cent or less than 0.5 gm. per day). In *diabetes mellitus* and other diseases where glucosuria occurs, the per cent of glucose in the urine may exceed 10 per cent with a daily excretion of 500 or even 1000 gms. Temporary glucosuria may be produced by ingestion of phloridzin (p. 571), various alkaloids, potassium chlorate and other compounds.

Glucose occurs most abundantly in the animal kingdom in the form of its condensation product glycogen.

**Glycogen.**§ — $(C_6H_{10}O_5)_n$ is a constituent of all growing animal cells. It is usually regarded as a reserve product, playing the same rôle in the animal economy as starch in the vegetable. The surplus carbohydrates of the food are stored up in the body as glycogen, about one-half of this being deposited in the liver and one-half in the muscles and other organs.

The percentage of glycogen in fresh meat and muscles varies from a trace to nearly 3 per cent; it is found in largest amount in the liver where it may constitute over 10 per cent of the weight of this organ. The amount of glycogen in the liver is subject to considerable fluctuation being greatest after a meal rich in carbohydrates and lowest after long abstinence from food. It is only after several weeks' fasting, however, that glycogen disappears completely from the liver. In its removal from the various organs of the body glycogen is hydrolyzed by enzymes to glucose, which is then transported by the blood to the different parts of the organism.

*Preparation of Glycogen.* — Glycogen is prepared by cooking finely divided livers with 60 per cent potassium hydroxide solution for two

* Bauer, J. prakt Chem., [2] **34,** 46.

† Winterstein, Ber., **27,** 3113.

‡ Errera, Compt. rend., **101,** 253.

§ Discovered by Claude Bernard in 1855 (Compt. rend., **41,** 461; **44,** 1325; **48,** 884, etc.). For a full account of this carbohydrate consult Pflüger's book "Das Glykogen," 2nd. Edit. (1906), Bonn.

hours. The filtrate is then diluted to 15 per cent potassium hydroxide and, after settling, the clear solution is mixed with 1 volume of 96 per cent alcohol. The precipitated glycogen is washed with a mixture of 1 volume of 15 per cent potassium hydroxide, and 2 volumes of 96 per cent alcohol. The crude product thus prepared is purified by dissolving in strong potassium hydroxide solution and reprecipitating with alcohol.

*Properties of Glycogen.* — Glycogen is obtained as an amorphous snow-white substance. The product is usually more or less hydrated so that it is difficult to prepare a substance of uniform composition. Glycogen dissolves in water to a faintly opalescent colloidal solution; it is also dissolved by hot alcohol; the best solvent is aqueous potassium hydroxide solution. Glycogen is strongly dextrorotatory, $[\alpha]_D$ = about +200. It is decomposed by butyric acid bacteria and other organisms, but is not fermented by yeast. Plant enzymes of different origin convert glycogen, some into maltose and some into d-glucose; the enzymes of the body, however, seem to hydrolyze glycogen only into d-glucose. Glycogen is hydrolyzed by acids into d-glucose in the same manner as starch, dextrin, and other polysaccharides of the glucose class.

Glycogen does not reduce Fehling's solution. Treated with iodine solution it gives a color varying from brown to wine-red, which disappears upon heating to 60° C. but returns again upon cooling.

Of other animal products, which may be regarded as glucose derivatives, may be mentioned *tunicin*,* or *animal cellulose* $(C_6H_{10}O_5)_n$, found in the outer membranes of Tunicates and other animals, and the so-called *animal gum* found by Landwehr † in various tissues and organs of the body.

**The Gluco-proteids.** — A number of animal substances, which are not of a pure carbohydrate nature, yield glucose as one of their hydrolytic products. Among these substances may be mentioned different nucleo-proteids, various albumins and certain mucins or mucoids. The chemistry of these products, however, is still unsettled, and it is uncertain whether the sugar derived from them consists of glucose alone or of a mixture of sugars.

**Honey.** — Another animal product rich in glucose is honey, although the sugar in this instance is primarily of vegetable origin being derived by bees and other insects from the nectar of flowers and other plant juices. Honey contains usually about 35 per cent of glucose; strained honey will frequently granulate and even solidify owing to crystallization

* Berthelot, Compt. rend., **47,** 227. Winterstein, Z. physiol, Chem., **18,** 46–56.

† Z. physiol. Chem., **8.** 122; **9,** 367; **18,** 193; **19,** 339; **20,** 249.

of its glucose. The granulation of honey was known to the ancients, and crystallized glucose as thus observed was probably the first sugar known to mankind.

**Synthesis of Glucose.** — d-Glucose has been synthetised in a number of ways. It has been prepared from d-mannose, by oxidizing this sugar to d-mannonic acid and converting the latter by molecular rearrangement (p. 775) into d-gluconic acid, whose lactone upon reduction gives d-glucose. The sugar has also been built up synthetically by Fischer * by condensation of formaldehyde to d, l-fructose, which upon reduction gives d, l-mannite and this upon oxidation d, l-mannonic acid. The latter is then resolved into its d- and l-components and d-glucose is prepared from the d-mannonic acid as first described.

**Preparation of Glucose.** — d-Glucose can be prepared directly from granulated honey by stirring the mass with a little 50 per cent alcohol and filtering upon a suction plate. The sugar thus obtained is purified from fructose, honey dextrin and other impurities by recrystallization.

In preparing glucose upon a large scale hydrolysis of some one of its natural condensation derivatives is employed. For this purpose starch, the raw material from which commercial glucose is made, is usually chosen.

*Preparation by Hydrolysis of Starch.* — One hundred grams of pure potato or corn starch are heated to boiling with 1000 c.c. of 2 per cent hydrochloric acid in a flask connected with a reflux condenser for 2 hours. The hot solution is neutralized with lead carbonate, cooled and filtered. The filtrate is evaporated to a thin sirup, shaken with an equal volume of hot 96 per cent alcohol and filtered from precipitated impurities. The alcoholic filtrate upon evaporation gives a sirup which rapidly crystallizes; the sugar thus obtained is purified by a second crystallization.

*Preparation by Hydrolysis of Cellulose.* — Glucose can also be prepared by hydrolysis of cellulose; 100 gms. of clean cotton or filter paper are slowly stirred into 500 c.c. of 80 per cent sulphuric acid. The mixture is allowed to stand 24 hours; it is then diluted to 5000 c.c. and heated in a boiling water bath for 5 hours. The hot solution is then neutralized with an excess of calcium carbonate, filtered and the filtrate concentrated to a sirup. The latter is then purified from gummy decomposition products by heating with alcohol and clarified by filtering through bone black. The alcoholic filtrate upon evaporation gives a sirup which soon crystallizes.

* Ber., **23**, 799.

*Preparation by Inversion of Sucrose.* — Glucose is also very easily prepared by inversion of cane sugar. For this purpose 1000 gms. of refined sugar are heated for 1 hour with 300 c.c. of water and 5 c.c. of concentrated hydrochloric acid in a flask immersed in a boiling water bath. The yellowish colored sirup is then poured into an evaporating dish and set aside in a cool place. Granulation will begin after a few weeks' standing when the glucose will separate as a thick or even solid mass of crystals. The latter are taken up with a little 50 per cent alcohol, filtered, washed with strong alcohol and recrystallized in the usual way.

The crystallization of glucose can always be hastened by priming its sirups with a small crystal of glucose from a previous preparation.

**Properties of Glucose.** — Glucose crystallizes both as the hydrate $C_6H_{12}O_6.H_2O$ and the anhydride. The hydrate is obtained by crystallizing from water at ordinary temperature and the anhydride by crystallizing from a hot saturated alcoholic solution. Glucose hydrate loses its water of crystallization between 50° and 60° C. To prepare anhydrous glucose from the hydrate the latter should be spread in a thin layer in a flat bottomed dish and heated at 60° C. until most of the water has been expelled; the temperature is then gradually raised to 100° C. when the sugar will be obtained perfectly granular and dry. Heating the hydrate directly to 100° C. will cause melting and when this occurs it is difficult to obtain a satisfactory preparation of anhydrous glucose.

Glucose anhydride consists of fine crystalline needles melting at 146° to 147° C. The sugar has a sweet taste, is very soluble in water (about 1 : 1 at 20°) and hot alcohol, but insoluble in ether. The constant rotation of the anhydride is $[\alpha]_D = +52.5$ (directly after solution $+106$ or more). For glucose hydrate $[\alpha]_D = +48.2$ (constant). Tanret * has considered glucose to exist in 3 modifications, a high-rotating form ($[\alpha]_D = +106$), a constant-rotating form ($[\alpha]_D = +52.5$) and a low-rotating form ($[\alpha]_D = +22.5$). Tanret's constant-rotating glucose is now considered to be simply an equilibrated mixture of $\alpha$-, or high-rotating, glucose and $\beta$-, or low-rotating, glucose. Reference has been made to these modifications under the subject of mutarotation.

**Fermentations of Glucose.** — Glucose undergoes a large number of fermentations a few of the more typical of which will be mentioned.

*Alcoholic Fermentation.* — Glucose is fermented to alcohol by many species of *Saccharomyces, Mucor, Torula, Mycoderma* and other organ-

* Compt. rend., **120**, 1060.

isms. The general formula for the fermentation of glucose to alcohol was first given by Gay-Lussac * as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = 2\ \underset{\text{Alcohol}}{CH_3CH_2OH} + 2\ \underset{\text{Carbon dioxide.}}{CO_2}.$$

or, 100 parts glucose = 51.11 parts alcohol +48.89 parts carbon dioxide. Pasteur,† however, showed that this formula was not absolutely correct, as he obtained under the best of conditions only 48.3 parts of alcohol and 46.4 parts of carbon dioxide from 100 parts of glucose. Pasteur obtained in addition to alcohol and carbon dioxide 2.5 to 3.6 parts of glycerol, 0.4 to 0.7 part of succinic acid and 1.3 parts of fat, cellulose and other substances, all of which he believed to be formed directly from the sugar. The latest researches, however, show that these minor products of fermentation are the result of metabolic processes within the yeast cells. The main phase of the fermentation is produced by the enzyme *zymase* which is secreted by the yeast. Buchner,‡ in fact, has separated zymase from crushed yeast and by its aid has fermented glucose into alcohol and carbon dioxide without the direct agency of the living cells.

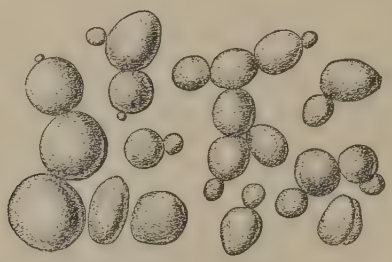


Fig. 193.—Saccharomyces cerevisiæ. After Hansen.

In the alcoholic fermentation of glucose a concentration of 5 to 20 per cent sugar gives the best results. The temperature should be maintained between 30° and 35° C., and a small amount of peptones, phosphates, tartrates and other salts be added as nutrient for the yeast. A growth of *Saccharomyces cerevisiæ*, or beer yeast, which is one of the best known alcohol-producing organisms, is shown in Fig. 193.

In addition to ethyl alcohol a number of other alcohols are pro-

* Ann. chim. phys. [1], **95,** 311.

† Ann. chim. phys. [3] **58,** 330, 355, 362.

‡ Ber., **30,** 117, 1110, 2668; **34,** 1523, etc. See also the book by Buchner and Hahn "Die Zymasegärung," Munich, 1903.

duced in the alcoholic fermentation of glucose; the most important of these are amyl, isobutyl and propyl alcohols with traces of hexyl alcohol and higher homologs. These higher alcohols (the fusel oil of distilleries) according to the researches of Ehrlich * are not formed from the sugar, however, but are secondary products derived from the amino acids of the yeast.

$$\underset{\text{Amino acid}}{R \cdot CHNH_2 \cdot COOH} + H_2O = \underset{\text{Higher alcohol}}{R \cdot CH_2OH} + \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Ammonia.}}{NH_3}.$$

*Lactic Fermentation.* — Glucose is fermented to lactic acid † by a large number of bacilli and bacteria. The formula for the fermentation of glucose into lactic acid in its simplest terms is expressed as follows:

$$\underset{\text{d-Glucose}}{C_6H_{12}O_6} = 2\ \underset{\text{Lactic acid.}}{CH_3\ CHOH\ COOH}.$$

The theoretical yield of lactic acid, however, is never obtained, more or less carbon dioxide, hydrogen, formic, acetic, butyric and propionic acids, mannite and other products of secondary or foreign fermentative origin being always formed.

In fermenting glucose to lactic acid a 10 per cent solution of the sugar is prepared (adding minute amounts of ammonium salts, nitrates, bran extract or other substances to serve as nutrients) and then sterilized. The cold solution is inoculated with a pure culture of *Bacillus lactis acidi* (or other lactic acid producing organism) and incubated at 45° to 55° C. for 3 to 6 days. A little powdered calcium carbonate is added from time to time to take up the excess of free acid which should be kept below 0.5 per cent, but never be entirely neutralized (otherwise the butyric fermentation may set in). If the lactic acid culture is pure, 98 per cent of the glucose may be converted into lactic acid by this method.

The lactic acid obtained by fermentation is usually optically inactive and consists of a racemic mixture of the d- and l-isomers. Certain organisms have been found, however, which seem to form the d-, or l-, acid alone or in excess. In these cases d, l-lactic acid may perhaps be formed first, the d-, or l-component being afterwards partly or wholly destroyed by secondary fermentation.

The conversion of d-glucose into lactic acid, according to Buchner,‡ is due to the action of a special enzyme secreted by the organisms.

*Butyric Fermentation.* — Glucose is fermented into butyric acid § by a number of species of bacteria; among the best known of these is

* Ber., **40**, 1027–47.
† Pasteur, Ann. chim. phys. [3], **2**, 257 (1842).
‡ Ber., **36**, 634.
§ Pasteur, Compt. rend., **52**, 344.

the *Clostridium butyricum*. The butyric fermentation, which proceeds only in the absence of air, follows approximately the following equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Butyric acid}}{CH_3CH_2CH_2COOH} + 2\,\underset{\text{Carbon dioxide}}{CO_2} + 2\,\underset{\text{Hydrogen.}}{H_2}$$

As by-products of the butyric fermentation there are generally formed butyl, ethyl and propyl alcohols, formic, acetic, propionic, valeric, caprylic, capric and lactic acids, and other substances either of secondary or foreign fermentative origin.

The butyric acid producing bacteria grow best at a temperature between 35° and 40° C. and require a medium perfectly free from acid. To secure the latter condition the butyric fermentation is carried out in presence of an excess of calcium carbonate which combines with the free acid as fast as it is formed.

*Viscous Fermentation.** — Glucose is fermented by a large number of different organisms to a mucilaginous gummy substance known as dextran. The gum which is formed during fermentation is probably a secondary product, being a constituent of the gelatinous capsule which encloses the organism. Among the best-known organisms, which produce the viscous fermentation, is the *Leuconostoc mesenterioides* † (Fig. 196) to which class belong also the *Bacterium pediculatum* ‡ (Fig. 197) and other gum-forming organisms found in sugar factories. The bacteria which form gum thrive best in the absence of air, and at a temperature varying from 30° to 35° C.

*Dextran*§, the gum of the viscous fermentation, and the cause of ropiness in wine and of the so-called "frog-spawn" in sugar factories, is precipitated from solution by means of alcohol. It is purified by dissolving in dilute sodium hydroxide, filtering and reprecipitating with alcohol acidified with acetic acid. The product, after drying and pulverizing, is obtained as a white powder, having the general formula $(C_6H_{10}O_5)_n$, and yielding glucose upon hydrolysis with sulphuric acid. The gum swells up in water but does not form a perfectly transparent solution except upon addition of a little alkali. Dextran is strongly dextrorotatory, $[\alpha]_D$ = about +200.

*Oxidizing Fermentations of Glucose.* — There are a large number of fermentations of glucose, which differ from the types previously de-

* Pasteur, Bull. soc. chim. (1861), 30.

† Van Tieghem, J. fabric. sucre, **20**, 30, 32.

‡ Alfred Koch and Hosaeus, Chem. Centralbl., (1894), II, 703.

§ For the copious literature upon dextran see references in Lippmann's "Chemie der Zuckerarten," 427–430.

scribed, in that oxygen is absorbed from the air with the formation of different acids. Such fermentations belong to the strictly aërobic class. The following are examples:

*Gluconic Acid Fermentation.* — d-Glucose is fermented to d-gluconic acid by the *Micrococcus oblongus*,* *Bacterium xylinum*† and numerous other organisms. In its simplest phase the reaction proceeds as follows:

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ CHO \\ \text{d-Glucose} \end{array} + O \quad = \quad \begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ COOH \\ \text{d-Gluconic acid.} \end{array}$$

With some organisms the fermentation proceeds almost quantitatively according to this reaction. In other cases the gluconic acid itself undergoes oxidation so that the theoretical yield is much reduced.

*Citric Acid Fermentation.* — Various organisms belonging to the *Citromyces* group ferment glucose into citric acid. The general formula for the reaction is given as follows:

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ CHO \\ \text{Glucose} \end{array} + 3O \quad = \quad \begin{array}{l} CH_2 - COOH \\ | \\ C(OH) - COOH \\ | \\ CH_2 - COOH \\ \text{Citric acid.} \end{array} + 2\,H_2O$$

The citric acid fermentation is attended as the formula shows by a high consumption of oxygen, about 200 c.c. of oxygen being taken up from the air for every gram of glucose fermented.

In fermentation experiments the yield of citric acid from glucose has not been found to exceed 50 per cent, owing to the fact that the citric acid itself undergoes oxidation in the later stages of the fermentation.

*Oxalic Acid Fermentation.* — Glucose is fermented into oxalic acid by a large number of moulds and bacteria. The complete conversion of glucose in this direction, however, is never reached, as the oxalic acid at a certain point of concentration begins to exercise a toxic effect upon the growth of the organisms. The oxalic acid, which is formed by the action of microörganisms, is probably a respiration product given off by the living cell rather than a true fermentation derivative (such as alcohol) formed from the sugar by the action of a special enzyme. The same is no doubt also true of many other acids.

*Other Acid Fermentations.* — Glucose is subject to a large number

* Boutroux, Compt. rend., **91**, 236.

† Brown, J. Chem. Soc., **49**, 432; **50**, 463.

of other fermentations in which acetic, propionic, formic, succinic and other acids are formed. In some of these cases the fermentation seems to be of a true enzymic character. Certain bacteria, for example, are able to convert glucose directly into acetic acid.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = 3\,\underset{\text{Acetic acid.}}{CH_3COOH}$$

Buchner and Meisenheimer* have been able to show that this change in certain fermentations is produced by a specific enzyme which they found possible to isolate. Many investigators believe that all other acid fermentations of glucose are also the result of special oxidizing enzymes or oxidases; this view, however, requires considerable more experimental proof before it can be accepted.

*Ester Fermentation.* — In many fermentations of glucose, as by the *Bacillus suavolens*, the production of alcohol and acids proceeds simultaneously, the result being the formation of different fruity esters, such as ethyl acetate, ethyl butyrate and ethyl valerate.

The chemistry of the different compounds, which are formed in the fermentation of glucose and other sugars, is so extensive that the student is referred for further particulars to the special works upon the subject.†

**Action of Alkalies upon d-Glucose.** — The products obtained by heating d-glucose with caustic alkalies have been briefly referred to. The chief product of this decomposition is d, l-lactic acid, the occurrence of which in molasses is due to the action of lime during clarification upon glucose and fructose. The lactic acid is easily obtained from molasses by acidifying with sulphuric acid and extracting with ether.

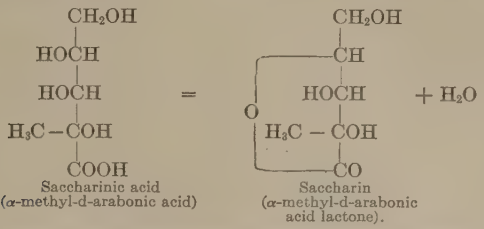
*Saccharin.* — Another alkali-decomposition product of glucose, which is found in sugar-house products, is *saccharin*, $C_6H_{10}O_5$, the lactone of saccharinic acid $C_6H_{12}O_6$. Saccharin is prepared according to Scheibler by dissolving 1 kg. of d-glucose (or d-fructose) in 7 to 8 liters of water, and adding, with continuous boiling, freshly slacked milk of lime, so that the solution is still alkaline after 3 to 4 hours. The cooled solution is saturated with carbon dioxide and the filtrate treated with oxalic acid just sufficient to combine with all the lime. The filtrate containing the saccharinic acid is evaporated to a sirup, which after long standing deposits crystals of saccharin.

Saccharinic acid, which is an isomer of glucose, is the $\alpha$-methyl

* Ber., **36**, 634.

† Particularly Lippmann's "Chemie der Zuckerarten" and Lafar's "Technische Mykologie."

derivative of d-arabonic acid, whose structural formula and that of saccharin are as follows:

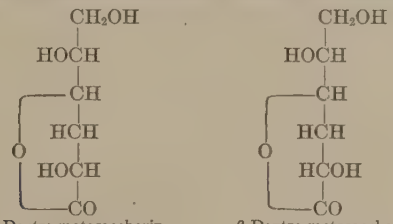



Saccharinic acid (α-methyl-d-arabonic acid)

Saccharin (α-methyl-d-arabonic acid lactone).

Saccharin forms clear rhombic double-refracting crystals which melt at 160° C. and sublime without decomposition. It is easily soluble in water, alcohol and ether, is not fermented by yeast, and does not reduce Fehling's solution. The specific rotation is $[\alpha]_D = +93.5$.

Saccharin is a type of a large group of isomeric substances * (isosaccharins, metasaccharins, etc.) which are produced by the action of alkalies upon different sugars. Nef by the action of 8 normal sodium hydroxide upon d-glucose obtained α- and β- dextro-metasaccharins.




α-Dextro-metasaccharin
$[\alpha]_D = +25.28$
m.p. = 104° C.

β-Dextro-metasaccharin
$[\alpha]_D = +8.2$
m.p. = 92° C.

**Tests for d-Glucose.** — No single sugar has been subjected to such a variety of tests as d-glucose, and the various compounds which have been obtained in its combination with different reagents number many hundred.

Glucose in its reduction of alkaline solutions of copper, silver, mercury and other metals, in its color reactions and many other tests behaves in the same way as other sugars of the aldose class. The following reactions are given as among the more characteristic of glucose and its derivatives.

*Saccharic Acid Test for Glucose.* — Glucose and the various substances which yield glucose upon hydrolysis are oxidized by strong

* For further particulars upon the different saccharins see Kiliani (Ber., **15,** 701, 2953; **16,** 2625; **18,** 631, 2514) and Nef (Ann., **376,** 1).

nitric acid to saccharic acid, which is recognized by means of its acid potassium or silver salt. The test, according to Tollens and Gans,* is best carried out as follows:

Five grams of the material to be examined are treated in a porcelain dish with 30 c.c. of nitric acid of 1.15 sp. gr. and the mixture evaporated with constant stirring upon a boiling water bath until evolution of red fumes has ceased and the resulting sirup has just begun to take on a permanent yellow color. The sirup is then taken up with a little water, heated over a flame and powdered potassium carbonate added until a drop of the brownish colored solution gives a blue reaction with red litmus paper. Glacial acetic acid is then added drop by drop until the mixture gives off a strong odor of acetic acid. If glucose was present in the original substance crystals of acid potassium saccharate will usually soon separate; if crystallization does not take place after a few hours' standing, as may happen when only small amounts of glucose are present, the sirup should be concentrated further by gentle evaporation. After 24 hours' standing the crystals, which have formed, are filtered off, or drained upon unglazed porcelain, and then recrystallized from the smallest possible amount of hot water. A third crystallization, using bone black, will usually eliminate the last traces of oxalic acid and other impurities and give a perfectly pure salt. The yield of acid potassium saccharate by this method is about 30 to 40 per cent of the original amount of glucose. The compound consists of shining rhombic crystals with characteristic trapezoidal faces, the appearance of which under the microscope is unmistakable. Acid potassium saccharate has the formula $COOH\ (CHOH)_4\ COOK$.

The acid potassium saccharate as above prepared can be further identified by conversion to the silver salt. For this purpose the acid potassium salt, after drying and weighing, is dissolved in a little water, to which ammonia is then added to the point of neutrality. The solution is then poured into a cold silver nitrate solution containing $AgNO_3$ to the amount of $1\frac{1}{2}$ the weight of the acid potassium salt taken. The precipitated saccharate of silver after standing a short time is filtered, washed free from silver nitrate and then dried in a dark place over concentrated sulphuric acid. The silver saccharate has the formula $C_6H_8O_8Ag_2$ and upon ignition in a porcelain crucible should show 50.91 per cent Ag.

In making the saccharic acid test for glucose it should be remembered that d-gluconic, d-glucuronic and d-gulonic acids and d-gulose also give saccharic acid upon oxidation with nitric acid. This limita-

* Ber., **21**, 2149.

tion, however, is a comparatively slight one and the saccharic acid reaction upon the whole is one of the best tests for d-glucose in presence of other sugars.

*Hydrazones and Osazones of Glucose.* — d-Glucose forms a large number of hydrazones with phenylhydrazine and its substituted derivatives. With phenylhydrazine itself two isomeric hydrazones are formed, one modification melting at 144° to 146° C. and the other at 115° to 116° C. The exact conditions under which these two isomers are formed and their structural relationship are not fully understood (see p. 359).

Fischer * and Stahel † recommended the diphenylhydrazone as one of the best compounds for identifying glucose. To carry out the test 1 part of sugar is dissolved in as little water as possible and 1.5 parts of diphenylhydrazine in alcoholic solution are added; if the mixture is not clear a little water or alcohol is added until the turbidity disappears. By allowing the mixture to stand several days the diphenylhydrazone $C_6H_{12}O_5 : N - N(C_6H_5)_2$ will separate as small colorless prisms, which, after recrystallizing from hot water, should melt at 161° to 162° C. By heating the mixture of sugar and diphenylhydrazine in a flask attached to a reflux condenser the formation of the hydrazone is completed within 2 hours. Upon evaporating the alcohol and taking up the uncombined hydrazine with ether the hydrazone will quickly separate. The glucose-diphenylhydrazone is insoluble in ether and addition of the latter will hasten crystallization. The diphenylhydrazone reaction offers an easy means for separation of d-glucose from d-fructose and other sugars. In case of a mixture of sugars it is advisable to conduct the reaction in the cold. It should be remembered in making the test that arabinose also gives a characteristic insoluble hydrazone with diphenylhydrazine; the two compounds can easily be separated, however, by crystallization and are readily distinguished from one another by their differences in melting point and composition.

Glucose-benzhydrazone ‡ $C_6H_{12}O_5 : N - NH \cdot COC_6H_5$ and methylphenylhydrazone § $C_6H_{12}O_5 : N - NCH_3 \cdot C_6H_5$ have also been employed for the separation and identification of glucose. Glucose may be separated from its hydrazones by decomposing the latter with benzaldehyde or formaldehyde as described on page 348. The free sugar, which is thus liberated, may be crystallized and further identified by determining its specific rotation.

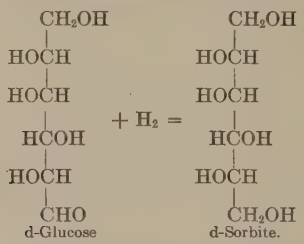
The best-known and earliest studied hydrazine derivative of glucose

* Ber., **23,** 805.
† Ann., **258,** 242.
‡ Wolff, Ber., **28,** 160.
§ Neuberg, Ber., **35,** 965.

is the phenylosazone $C_6H_{10}O_4(:N-NH\cdot C_6H_5)_2$. The compound is obtained as yellow needle-shaped crystals by heating 1 part glucose, 2 parts phenylhydrazine chloride and 3 parts sodium acetate in 20 parts of water. The osazone is purified by recrystallizing from alcohol, and should have a melting point of 205° to 206° C. (capillary tube method).

The osazone reaction of glucose is of but little value as a means of identification, owing to the fact that d-mannose and d-fructose both give the same compound.

*Reduction of Glucose.* — d-Glucose upon treatment with sodium amalgam in acid solution is reduced to the alcohol d-sorbite.



d-Glucose d-Sorbite.

If the reaction is allowed to proceed in alkaline solution mannite is also formed, owing to rearrangement of a part of the glucose into d-mannose and d-fructose previous to reduction.

Glucose is oxidized in aqueous solution by means of bromine to gluconic acid $CH_2OH(CHOH)_4COOH$, which upon evaporation gives the lactone $C_6H_{10}O_6([\alpha]_D$ after solution $= +\ 68.2)$.

The reactions of glucose with benzoyl chloride, formaldehyde, acetic anhydride, mercaptan, etc., have already been referred to.

**The Synthetic Glucosides.** — Reference has already been made to the combination of reducing sugars with alcohols. These compounds, while of little analytical importance, have considerable theoretical interest, and the following description is given of the methyl derivatives of glucose.

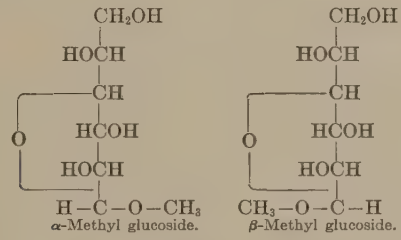
The combination of d-glucose and methyl alcohol, according to Fischer's * early method, is accomplished by dissolving glucose in cold methyl alcohol and saturating the solution with dry hydrochloric acid gas. Crystals of the $\alpha$-methyl glucoside are obtained upon evaporating the solution; the mother liquor contains a second isomeric $\beta$-methyl glucoside, which can be isolated by careful fractional crystallization.

* Ber., **26,** 2400; **28,** 1151.

The $\alpha$- and $\beta$-methyl glucosides, although having the same general formula, $C_7H_{14}O_6$, show a marked difference in certain of their properties, as is seen from the following:

| | $\alpha$-Methyl glucoside. | $\beta$-Methyl glucoside. |
|---|---|---|
| Melting point | 165°–166° C. | 108°–110° C. |
| Specific rotation | +158 | −32 |
| Action of maltase | Hydrolyzing | Non-hydrolyzing |
| Action of emulsin | Non-hydrolyzing | Hydrolyzing |

Following the suggestion of Simon * the $\alpha$- and $\beta$-methyl glucosides of high and low rotation are usually regarded as respective compounds of the high rotating $\alpha$-glucose and low rotating $\beta$-glucose. Armstrong,† in fact, showed by polariscopic measurements that $\alpha$-methyl glucoside was resolved by the enzyme *maltase* (*$\alpha$-glucase*) into $\alpha$-glucose of high initial rotation and $\beta$-methyl glucoside by the enzyme *emulsin* (*$\beta$-glucase*) into $\beta$-glucose of low initial rotation. Adopting the formulæ given on page 192 for $\alpha$- and $\beta$-glucose, the configurations of $\alpha$- and $\beta$-methyl glucoside would be.



$\alpha$-Methyl glucoside. $\beta$-Methyl glucoside.

Testing the hydrolyzing power of maltase or emulsin upon the glucosides, disaccharides and other natural condensation products of glucose has been used by Armstrong ‡ as a means of determining whether the glucose in a given combination belongs to the $\alpha$- or $\beta$-form.

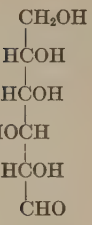
Compounds resembling the $\alpha$- and $\beta$-methyl glucosides have been prepared by condensing glucose with other alcohols. The compounds have usually a sweet taste, do not reduce Fehling's solution, and exhibit none of the other aldehyde properties peculiar to glucose.

* Compt. rend., **132,** 487, 596.

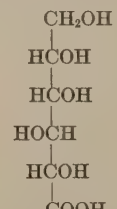
† J. Chem. Soc., **83,** 1305.

‡ Armstrong's " The Simple Carbohydrates and Glucosides " (1910), 43.

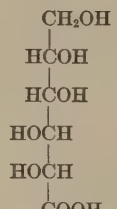
**l-Glucose.** —



l-Glucose has not been found free in nature and possesses only a theoretical interest from the relationship to its d-isomer. The sugar has been prepared synthetically * from the pentose sugar l-arabinose. The latter upon treatment with hydrocyanic acid and saponification of the addition product (Kiliani's cyanhydrine synthesis) gives a mixture of the two hexonic acids.



l-Gluconic acid.



l-Mannonic acid.

The l-gluconic and l-mannonic acids are separated by crystallization of their lactones. The lactone of l-gluconic acid is reduced by sodium amalgam to l-glucose.

*Properties.* — l-Glucose consists of small prismatic crystals melting ing at 141° to 143° C. In its outward properties the sugar resembles d-glucose. Its specific rotation was found by Fischer to be $[\alpha]_D = -51.4$; mutarotation was present, the reading after solution being − 95.5.

l-Glucose is not fermented by yeast and in this respect shows an important difference from d-glucose.

Nitric acid oxidizes l-glucose to l-saccharic acid whose acid potassium and silver salts resemble those of d-saccharic acid.

Diphenylhydrazine forms with l-glucose a characteristic hydrazone, which melts at 162° C. and is in other respects very similar to d-glucose-diphenylhydrazone. The phenylosazone of l-glucose is formed under the same conditions as d-glucose-phenylosazone and resembles the latter in melting point, crystalline form and solubility; it is readily dis-

* Fischer, Ber., **23**, 2611.

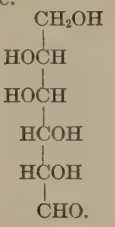
tinguished, however, from the d-glucose-osazone by its dextrorotation in glacial acetic acid solution.

**d, l-Glucose.** — Racemic glucose was obtained by Fischer,* as a sweet colorless inactive sirup, by dissolving equal parts of d- and l-glucose in water and evaporating.

d, l-Glucose gives with diphenylhydrazine a colorless diphenylhydrazone of melting point 132° to 133° C., which is 30° C. below the melting point of either d- or l-glucose-diphenylhydrazone. Oxidation of d, l-glucose with nitric acid gives d, l-saccharic acid, whose acid-potassium salt is very similar to those of its d- and l-components.

Racemic glucose is easily resolved by means of yeast, the d-glucose being completely fermented and the l-glucose remaining behind.

**D-MANNOSE.** — Seminose.




**Occurrence.** — d-Mannose is found in the free condition according to different investigators in the juices of various plants and in many germinating seeds. The sugar has also been found in certain molasses from tropical cane-sugar factories; the mannose in molasses, however, is not derived from the cane but is formed by the action of the lime used in clarification upon the glucose and fructose of the cane juice.

d-Mannose is most widely distributed in nature as an anhydride condensation product *mannan* which in its simple or complex form makes up one of the most abundant of the hemicelluloses.

**Mannan** $(C_6H_{10}O_5)_n$. — This carbohydrate, either in its simple form or as one of the so-called "paired mannans" (glucomannan, galactomannan, fructomannan, etc.), occurs in nearly all plants from the simplest unicellular Protophytes to the most highly developed Phanerogams. It is found in the cellular matter of yeast, in different moulds, in various algæ, in plant gums, in the wood, bark and roots of many trees, and in bulbs, nuts, grains, seeds, fruits, berries, leaves and other plant tissues.

* Fischer, Ber., **23**, 2611.

*Yeast Mannan.* — A convenient material for preparing one of the mannans is pressed yeast (manufactured without starch). Salkowski's * method of separating and purifying yeast mannan, or yeast gum, is as follows: 500 gms. of yeast are boiled gently for one-half hour with 5 liters of 3 per cent potassium hydroxide. The solution is then allowed to stand, and the clear liquid, which is poured off, heated with 750 c.c. of Fehling's solution upon a water bath. The yeast gum is thrown out as a bluish white insoluble copper compound, which is purified from its mother liquor by boiling and squeezing out with water. The copper compound is then decomposed by rubbing up with a slight excess of hydrochloric acid and 4 to 5 volumes of 90 per cent alcohol added. The acid alcoholic solution is poured off from the precipitated gum; the latter is then dissolved in water and reprecipitated by alcohol as before. After washing with a little absolute alcohol and ether, the gum is redissolved in 25 parts of water, a few drops of hydrochloric acid are added and the solution poured into 7 volumes of absolute alcohol. The precipitated gum is washed with alcohol and ether and then allowed to remain under ether until the plastic mass hardens to a brittle solid. The ether is then poured off, the gum ground up in a mortar and dried over sulphuric acid.

Yeast mannan consists of a white non-hygroscopic powder, easily soluble in warm water to a clear limpid solution and showing a specific rotation of $[\alpha]_D = +\ 90.1$. Hydrolysis of yeast gum with hydrochloric or sulphuric acids gives large amounts of d-mannose. According to Oshima d-glucose and fucose are also formed, so that yeast gum in all probability belongs to the complex mannans.

*Salep Mannan.* — A very pure mannan has been prepared from the mucilage of salep, a drug consisting of the dried decorticated tubers of different orchidaceous plants. Salep yields from 40 to 50 per cent of mucilage; the latter when separated from insoluble matter consists almost wholly of a water soluble mannan, which can be precipitated from solution by means of alcohol. Salep mannan † has the general formula $(C_6H_{10}O_5)_n$ and is hydrolyzed by acids first to lower mannosaccharides and finally to d-mannose.

Mannans have been isolated from many other plant substances, the general method of preparation consisting in the extraction of the material with hot 2 to 3 per cent alkali and precipitation of the gum with Fehling's solution as described under the method for yeast gum. The combining power of Fehling's solution with mannan is very marked,

* Ber., **27,** 497, 925.

† Hilger, Ber., **36,** 3198.

the reagent causing a precipitation of 1 part mannan in 1000 parts of liquid.

**Preparation of Mannose.** — Mannose may be prepared either by hydrolysis of yeast gum, salep mucilage or any other of the isolated mannans, or by direct hydrolysis of some plant material rich in mannan. The latter method is the most direct and the easiest to carry out, there being several common vegetable substances, such as ivory nuts, carob beans, coffee berries, date seeds, etc., which yield large amounts of mannose upon hydrolysis. Ivory nuts, or vegetable ivory (the fruit of *Phytelephas macrocarpa*), which is used so extensively for making buttons, is one of the best substances for preparing mannose. The method of Fischer and Hirschberger * is as follows:

*Preparation of d-Mannose from Ivory Nuts.* — One part of ivory-nut shavings (from button factories) is heated with 2 parts of 6 per cent hydrochloric acid in a boiling water bath for 6 hours in a flask connected with a reflux condenser. The hot solution is then pressed out from the insoluble residue and the latter treated with a little water and repressed. The combined extract is then neutralized with sodium hydroxide, decolorized with bone black, filtered and treated in the cold with an excess of phenylhydrazine (0.3 gm. for every 1 gm. of ivory-nut shavings) dissolved in acetic acid. The very insoluble mannose-hydrazone soon separates as a thick crystalline precipitate, which is filtered off after 24 hours, washed with cold water and dried. The hydrazone may be purified by recrystallizing from a large volume of hot water or from 50 per cent alcohol containing a little pyridine, but for the purpose of preparing mannose the troublesome recrystallization may be dispensed with.

Mannose may be separated from its hydrazone by any of the methods previously described (p. 348). The best procedure according to Tollens † is the following: 50 gms. of mannose hydrazone, 40 gms. of benzaldehyde, 50 gms. of alcohol and 50 gms. of water are heated upon the water bath for about one-half hour in a flask connected with a reflux condenser. The solution is then cooled and filtered from the insoluble benzaldehyde-hydrazone; the filtrate is shaken out with ether, decolorized with bone black, filtered and evaporated to a sirup. The latter rarely crystallizes until it has been primed with a crystal of mannose from a previous preparation.

Crystallized mannose has been prepared by van Ekenstein ‡ by dis-

* Ber., **22,** 3218.

† Abderhalden's "Biochem. Arbeitsmethoden" (1909), II, 74.

‡ Rec. trav. Pays-Bas, **14,** 329; **15,** 222.

solving mannose sirup in methyl alcohol, adding a half volume of ether and after 24 hours decanting from the sirupy precipitate. The methyl-alcohol-ether solution on long standing will deposit crystals of mannose, which may be used for priming impure sirups.

After the mannose-containing sirup has crystallized, the sugar is freed from its mother liquor by spreading upon porous plates and then purified by recrystallizing.

According to Neuberg and Mayer * mannose is best separated from its hydrazone by means of formaldehyde. The sugar crystallizes at once in a highly pure condition without a trace of decomposition products.

*Preparation of d-Mannose from Carob Beans.* — d-Mannose has been obtained by Herissey † from the seeds of the carob bean (St. John's bread) by allowing the mannan of the seeds to react with the accompanying enzyme, *seminase:* 500 gms. of the finely ground seeds are treated with a solution of 60 gms. sodium fluoride in 4000 c.c. of water and allowed to stand at 33° to 35° C. The fluoride prevents fermentation by microörganisms, while the seminase of the seeds converts the mannan to d-mannose, which is precipitated from solution as the hydrazone according to the method described.

**Synthesis of d-Mannose.** — d-Mannose has been made synthetically in a number of ways. d-Mannite is oxidized by dilute nitric acid to d-mannose which can then be separated as the hydrazone. d-Mannose can also be formed from d-glucose and d-fructose, through molecular rearrangement by action of dilute alkalies (p. 303). The sugar has also been built up by Fischer ‡ from formaldehyde; the latter by condensation gives d, l-fructose, which upon reduction gives d, l-mannite, and this upon oxidation with bromine yields d, l-mannonic acid. The latter is resolved by crystallization of its strychnine salts into the d- and l-components. The lactone of the d-mannonic acid upon reduction gives d-mannose.

**Properties of d-Mannose.** — d-Mannose crystallizes as the anhydride $C_6H_{12}O_6$ in rhombic crystals melting at 132° C. The sugar has a pleasant sweet taste and is easily soluble in water and 80 per cent alcohol, very slightly soluble in hot absolute alcohol and insoluble in ether.

d-Mannose has a constant specific rotation of $[\alpha]_D = +14.25$; the initial rotation is to the left, $[\alpha]_D$ 3 minutes after solution being $-13.6$.

d-Mannose is fermented by yeast to alcohol and carbon dioxide in the same manner, but not with the same rapidity, as d-glucose. The

* Z. physiol. Chem., **37,** 547. † Compt. rend., **133,** 49, 302. ‡ Ber., **23,** 370.

sugar is also easily fermented by different lactic acid organisms. d-Mannose reacts with alkalies similarly to d-glucose.

**Tests for d-Mannose.** — d-Mannose is best recognized by means of its phenylhydrazone, $C_6H_{12}O_5 : N - NHC_6H_5$, to which repeated reference has been made. The compound crystallizes in colorless rhombic prisms, which melt upon slow heating at 186° to 188° C., but with rapid heating at 195° to 200° C. The hydrazone is almost insoluble in cold water, but is dissolved in 80 to 100 parts of hot water; it is but little soluble in concentrated alcohol, ether or acetone; the best solvent is hot 60 per cent alcohol. Dissolved in hydrochloric acid it exhibits levorotation.

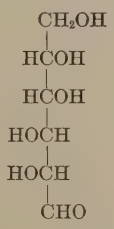
On long heating with phenylhydrazine, d-mannose, or its hydrazone, is converted into an osazone which is identical with that of d-glucose (p. 354).

d-Mannose upon reduction with sodium amalgam is converted into its alcohol d-mannite, which melts at 166° C. and in borax solution is dextrorotatory.

*d-Mannite* is very widely distributed in nature and has been found in ash manna, lilac leaves, mushrooms, sea algæ and in the olive, cactus, pineapple, onion and many other plants. The best natural source is ash manna, from which d-mannite is obtained by extraction with alcohol.

Oxidation of d-mannose with bromine in aqueous solution gives d-mannonic acid, $CH_2OH(CHOH)_4COOH$, whose lactone, $C_6H_{10}O_6$, is dextrorotatory ($[\alpha]_D = +53.8$). Upon oxidation with nitric acid d-mannose and d-mannonic acid give d-mannosaccharic acid, $COOH(CHOH)_4COOH$, whose double lactone, $C_6H_6O_6$, melts at 180° to 190° C. and has a rotation of $[\alpha]_D = +201.8$.

**l-Mannose.** —



l-Mannose has not been found in nature either in the free or combined form; it has been prepared synthetically * in several ways. The best starting point is l-arabinose, which, as shown under l-glucose, is converted by means of Kiliani's cyanhydrine reaction into both l-gluconic and l-mannonic acids. The lactone of the latter upon re-

* Fischer, Ber., **23**, 370.

duction with sodium amalgam gives l-mannose. The sugar can also be prepared by Fischer's synthesis from formaldehyde as described under the synthesis of d-mannose. l-Mannose has been obtained only in form of a colorless unfermentable levorotatory sirup.

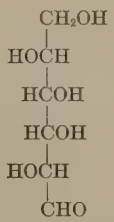
*Tests for l-Mannose.* — l-Mannose forms with phenylhydrazine an insoluble hydrazone, which resembles d-mannose-phenylhydrazone in melting point and other properties. The l-mannose-hydrazone, however, exhibits dextrorotation, when dissolved in hydrochloric acid, and this property serves to distinguish it from the d-mannose-hydrazone. The phenylosazone of l-mannose is identical with l-glucosazone.

l-Mannose upon reduction gives its alcohol l-mannite, which melts at 166° C. and in borax solution is levorotatory. Oxidation with bromine converts l-mannose to l-mannonic acid, whose lactone is levorotatory ($[\alpha]_D = -54.8$). Oxidation with nitric acid converts l-mannose and l-mannonic acid to l-mannosaccharic acid whose double lactone melts at 180° C. and has a rotation of about $[\alpha]_D = -200$.

**d, l-Mannose.** — Racemic mannose was obtained by Fischer * as a colorless inactive sirup by reduction of the lactone of d, l-mannonic acid. By decomposing d, l-mannose-phenylhydrazone with formaldehyde Neuberg and Mayer † obtained d, l-mannose in the form of crystals which melted at 132° to 133° C. d, l-Mannose forms an insoluble phenylhydrazone melting at 195° C.; its osazone is identical with d, l-glucosazone. The sugar upon reduction gives d, l-mannite; oxidation with bromine yields d, l-mannonic acid and with nitric acid d, l-mannosaccharic acid.

d, l-Mannose can be resolved by means of yeast which ferments only the d-mannose. Indirectly the sugar can be resolved by conversion to d, l-mannonic acid. The strychnine salt of the latter is then treated with boiling alcohol which dissolves only the salt of the d-acid. The lactones of the separated mannonic acids yield upon reduction the respective sugars, d- and l-mannose.

**D-GALACTOSE.** —



* Ber., **23**, 381. † Z. physiol. Chem., **37**, 545.

**Occurrence.** — Free d-galactose has been reported as occurring in the whey of milk and in the tissues of certain seeds during germination; the galactose thus found, however, is purely transitory, being derived by enzyme action from some of its higher condensation products, which, as glucosides, polysaccharides and hemicelluloses, are found widely distributed in nature.

d-Galactose occurs in the vegetable world as a constituent of many glucosides, in which compounds it is often united with other sugars. The glucoside *xanthorhamnin*, which gives d-galactose and rhamnose upon hydrolysis, has already been mentioned. In the same way the glucoside *digitonin*, a constituent of commercial digitalis, is hydrolyzed by heating with dilute acids into galactose and glucose.

$$\underset{\text{Digitonin}}{C_{27}H_{44}O_{13}} + 2\,H_2O = \underset{\text{d-Galactose}}{C_6H_{12}O_6} + \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Digitogenin.}}{C_{15}H_{24}O_3}.$$

*Convallarin* and *convallamarin* from *Convallaria majalis* (lily of the valley), *myrticolorin* from the leaves of *Eucalyptus macrorhyncha*, *sapotoxin* from certain species of Saponaria, and many other complex glucosides, whose constitution remains to be established, yield upon hydrolysis d-galactose, which is usually mixed with other sugars.

d-Galactose is also found united with other sugars as a constituent of different higher saccharides, such as lactose, melibiose, raffinose, rhamninose and stachyose.

**Galactans.** — d-Galactose is found most widely distributed in the vegetable kingdom as a constituent of many gums, hemicelluloses, mucilages, pectins and other plant materials. In these cases the galactose usually exists as an anhydride condensation product or galactan. The galactans make up a numerous group of substances, the exact constitution of which has not been thoroughly established. In the galactans d-galactose shows the same tendency to form combinations with other sugars as was noted in the case of its glucosides and polysaccharides; there are arabogalactans, xylogalactans, mannogalactans, glucogalactans and other combinations each of which shows well-marked differences in behavior towards alkalies and other reagents.

China moss, Ceylon moss, Irish moss, Iceland moss and many other plants belonging to the algæ, mosses and lichens yield mucilages, which are dissolved by hot water and precipitated therefrom by alcohol. The substances thus prepared consist mostly of galactan and are hydrolyzed by hydrochloric or sulphuric acid to d-galactose. Oxidation with nitric acid produces large quantities of mucic acid.

*Arabogalactans* or *galactoarabans* are found in the seeds of lupines,

beans, peas and other legumes * in amounts varying from 5 to 20 per cent. The cellular tissues of the crushed seeds are extracted successively with water, alcohol, ether and 0.2 per cent potassium hydroxide; the residue from this treatment consists largely of galactoaraban. The crude product is purified by dissolving in hot 2 per cent potassium hydroxide and precipitating the clear solution with strong alcohol, which throws out the galactoaraban as a yellowish colored potassium compound. The latter is washed with alcohol, decomposed with dilute acid and the pure gum precipitated by addition of strong alcohol.

Galactoarabans have also been found in plant exudations and gums, in vegetable mucilages, in the slimy envelopes of different bacteria, in unripe sugar-beets and in many other products. The galactoarabans are easily hydrolyzed by hydrochloric and sulphuric acids into d-galactose and l-arabinose. In many cases this hydrolysis can be effected by diastase and other enzymes; the latter in the process of germination no doubt convert the galactoaraban of seeds into sugars which are then assimilated by the growing embryo. The galactoarabans yield mucic acid upon oxidation with nitric acid, and furfural upon distillation with strong hydrochloric acid.

*Galactoxylan* † has been found as a gummy constituent of the cellular tissues of wheat, barley and other grains, and also appears to occur in various complex gums. Galactoxylan is hydrolyzed by dilute acids to galactose and xylose. Mucic acid is obtained upon oxidation with nitric acid, and furfural upon distillation with hydrochloric acid.

*Galactomannan* ‡ occurs as a constituent of many hemicelluloses; it has been found in the coffee berry, in cocoanuts, in the carob bean, in the seeds of *Strychnos Ignatii* (St. Ignatius' beans) and in other plant substances. Hydrolysis of galactomannan gives galactose and mannose.

Galactans of a more complex character than the above are also found in nature. Among such galactans may be mentioned flax-seed mucilage, which upon hydrolysis gives d-galactose, d-glucose, l-arabinose and l-xylose.

**The Pectins.** § — Closely related to the plant mucilages and gums are the pectins, an important group of substances widely distributed in nature. The pectins are found in apples, pears, grapes and most

* Schulze, Ber., **22,** 1192.

† Lintner and Düll, Z. angew. Chem. (1891), 538.

‡ Lippmann's "Chemie der Zuckerarten," p. 694.

§ For a fuller account of the pectins see article by Victor Grafe, "Biochemisches Handlexicon," pp. 80–94; also the early papers by Fremy to whom much of our knowledge is due (J. Pharm. [2], **26,** 368 (1840); Ann. chim. phys. [3], **24,** 5).

other fruits, in carrots, beets and other root organs, and in the tissues of many other plants, as the flax and hemp.

The pectins, which are soluble, are derived from an insoluble intercellular mother substance, called *pectose*, which is regarded by many chemists as an oxygen, or acid, derivative of cellulose. The conversion of pectose into pectin takes place in the ripening of fruits, and in the retting of flax and hemp; the process is attributed to the action of a special enzyme *pectosinase*.

A good material for preparing pectin is the juice of ripe pears. The juice is treated first with oxalic acid to break up lime compounds and then with tannic acid to precipitate albumin. The clarified juice is filtered and treated with an excess of strong alcohol, which precipitates the pectin. The latter is filtered off, purified by dissolving in water and reprecipitating with alcohol, and then dried over concentrated sulphuric acid. As thus prepared pectin consists of an amorphous white substance, which dissolves easily in water to a neutral solution. The pectins differ greatly in their optical properties; the pectin from orange skins, for example, is inactive, while the pectin from gooseberries gives $[\alpha]_D = +\ 194$.

Upon long boiling with water pectins and pectose are converted into *parapectin* of weak acid reaction. Boiling with dilute acids converts pectose, pectin and parapectin into *metapectin*, which has also the properties of a weak acid.

The pectins by the action of another enzyme *pectase* are converted into *pectic acids*, the calcium salts of which give fruit juices the property of jellifying. Pectic acids are also produced from pectose and pectin by boiling several hours with dilute alkali.

As precipitated from solutions of its salts pectic acid is obtained as a white amorphous jellylike mass, insoluble in water, alcohol and ether, but easily soluble in alkalies; $[\alpha]_D = +\ 186$ to $+\ 300$.

Pectose, pectin, parapectin, metapectin and pectic acid are converted by hot alkaline solutions into *parapectic acid*. The final product obtained by the action of alkalies upon the pectin substances previously named is *metapectic acid*, which is identical with *arabinic acid* described under l-arabinose.

The different pectin substances, which have been named, are all hydrolyzed by boiling with dilute mineral acids into d-galactose and l-arabinose, the yield of each sugar depending upon the nature of the product. The hydrolysis of the pectins into galactose and arabinose is also supposed by some to take place in nature through the agency of a third enzyme *pectinase*.

Oxidation of the pectins with nitric acid gives a large yield of mucic acid, and distillation with hydrochloric acid produces much furfural.

The chemistry of the pectins is still in a very unsettled condition. The neutral water-soluble pectins are usually regarded as lactones or esters of the various pectic acids, but the constitution of the latter, as well as that of the parent substance pectose, has not been determined.

d-Galactose occurs most widely in the animal kingdom as a constituent of milk sugar. It has also been recognized by different investigators among the saponification products of protagon, a constituent of nerve and brain tissue. The galactose in protagon is supposed to be part of an amino-phosphoric-fatty acid complex, the exact constitution of which is unknown. d-Galactose has also been reported to be present in different nucleo-proteids, mucins and other substances of animal origin, but the identity of the sugar in some of these cases has not been fully established.

**Synthesis of d-Galactose.** — The synthesis of d-galactose has been accomplished in several ways. It has been built up by Fischer and Ruff * from d-lyxose, which, by addition of hydrocyanic acid and saponifying (Kiliani's cyanhydrine synthesis), gives d-galactonic and d-talonic acids, the former, however, in much greater amount.† The lactone of d-galactonic acid upon reduction gives d-galactose.

d-Galactose has also been formed by Lobry de Bruyn and van Ekenstein ‡ by heating the ketose sugar l-sorbose with dilute alkalies, a mutual rearrangement taking place between these two sugars similar to that noted between d-glucose and d-fructose.

**Preparation of d-Galactose.** *From Milk Sugar.* — d-Galactose is most easily prepared by hydrolyzing milk sugar. For this purpose 1 part of milk sugar is heated with 10 parts of 2 per cent sulphuric acid in a boiling water bath for 4 hours; the free acid is then neutralized with an excess of calcium or barium carbonate and the solution filtered from the insoluble residue of sulphate and carbonate. The filtrate is evaporated to a sirup which will usually crystallize within a few days; crystallization may be hastened by priming the sirup with a crystal of galactose from a previous preparation. The impure galactose

* Ber., **33,** 2142.

† Fischer calls attention to the fact that in Kiliani's synthesis the yield of the two acids is never the same, one isomer being always produced in larger amount (Ann., **270,** 64).

‡ Rec. trav. Pays-Bas, **19, 1.**

from the first crystallization is filtered off, washed with a little 80 per cent alcohol and then redissolved in as little hot water as possible; hot strong alcohol is then added, the solution boiled with a little bone black and filtered; the filtrate upon cooling will soon deposit crystals of pure d-galactose.

*Preparation of d-Galactose from Agar-agar.* — d-Galactose may also be prepared by hydrolysis of plant materials rich in galactan, as Agar-agar. The latter when heated with 10 parts of 2 per cent sulphuric acid for 12 hours in a boiling water bath is largely hydrolyzed to d-galactose which may be crystallized by neutralizing the acid solution and evaporating to a sirup as described in the preceding method.

**Properties of d-Galactose.** — d-Galactose crystallizes from water as a monohydrate, $C_6H_{12}O_6.H_2O$, in the form of large prismatic needles, and from strong ethyl and methyl alcohols as the anhydride in the form of fine hexagonal crystals. The hydrate melts at 118° to 120° C. and the anhydride at about 165° C. The sugar has a sweet taste, is easily soluble in water, moderately soluble in 50 per cent alcohol, but practically insoluble in absolute alcohol and ether.

d-Galactose is strongly dextrorotatory; the value for constant rotation is about $[\alpha]_D = +81$, the figure being influenced both by temperature and concentration (see p. 181). The sugar shows strong mutarotation, $[\alpha]_D$ immediately after solution being about $+140$.

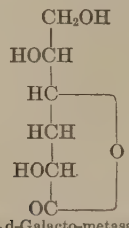
Tanret * has prepared d-galactose in a modification of low specific rotation. By dissolving 12 gms. of ordinary d-galactose in 30 gms. of water, adding 0.03 gm. of sodium phosphate exactly neutralized with sulphuric acid, heating a few minutes on the water-bath and then, after cooling, strongly agitating with 200 c.c. absolute alcohol, crystals of a galactose are obtained which give after solution a value for $[\alpha]_D$ of only $+53$; this low rotation, however, increases upon standing of the solution to the true constant value of ordinary galactose. The transition is effected at once by heating the solution or by adding a trace of alkali just as with the high rotating form of galactose.

**Action of Alkalies upon d-Galactose.** — By the action of dilute alkalies d-galactose is transformed into a mixture of isomeric hexoses, d-talose, d-tagatose, l-sorbose and galtose as described under these several sugars.

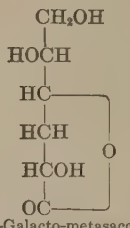
By the action of 8 normal sodium hydroxide Nef† obtained 40 to 45 per cent d, l-lactic acid, 10 per cent $\alpha$- d-galacto-metasaccharin, 5 to 10 per cent $\beta$- d-galacto-metasaccharin, 5 per cent $\alpha$-and $\beta$- d-isosaccharin, and numerous other saccharins. The two metasaccharins, which

* Bull. soc. chim. [3], **15**, 195. † Ann. **376**, 1.

are the most important of the group, have the following structural formulæ:



α-d-Galacto-metasaccharin.
$[\alpha]_D^{20} = -45.3$
m. p. = 144° C.



β-d-Galacto-metasaccharin.
$[\alpha]_D^{20} = -62.96.$
m. p. = 55° – 60° C.

The α-d-isosaccharin is described under lactose.

**Fermentation.** — d-Galactose is fermented by yeast in presence of suitable nutrients to alcohol and carbon dioxide; the fermentation proceeds, however, more slowly than with d-glucose and requires about 8 days for completion. The yield of ethyl alcohol with cultures of pure yeast is about 45 to 46 per cent of the weight of galactose taken. According to Buchner and Rapp * the alcoholic fermentation of d-galactose is due to the enzyme zymase. Different species of Mucor and other moulds also cause alcoholic fermentation of d-galactose.†

Many lactic acid producing organisms cause fermentation of d-galactose with formation of d, l-lactic acid; with some organisms the l-lactic acid is produced in greater amount.

*Bacterium xylinum*, the so-called sorbose bacterium, oxidizes d-galactose to d-galactonic acid.

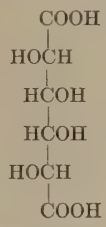
**Tests for d-Galactose.** *Mucic Acid Reaction.* — The test most generally employed for detecting d-galactose, either in the free or combined form, is the production of mucic acid upon oxidation with nitric acid. The reaction is carried out as described under the determination of galactan (p. 459). d-Galactose by this method ‡ yields over 75 per cent of its weight as mucic acid. It must be remembered that mucic acid is also formed by the oxidation of l-galactose, dulcite and d- and l-galactonic acids so that other reactions, such as the isolation of the sugar from its hydrazone, or the fermentation test, must be used for confirmation.

* Ber., **31**, 1090.

† The idea of Dubrunfaut that d-galactose was not fermented by yeast was shown by Pasteur to be erroneous. A later view of Bourquelot that d-galactose could be fermented only in presence of glucose, or some other easily fermentable sugar, was completely disproved by Tollens and Stone.

‡ Tollens and Kent, Ann., **277**, 222.

*Mucic acid* has the configuration:



It crystallizes in minute granular rhombic prisms, which melt on rapid heating at 212° to 215° C. and are almost insoluble in water (1 part in 300 c.c. cold water). Mucic acid, as is evident from its symmetrical structure, is optically inactive. Upon heating with concentrated hydrobromic acid, or other dehydrating agents, mucic acid is converted to dehydromucic acid (p. 781).

*Reactions of d-Galactose with Phenylhydrazine.* — d-Galactose when treated with phenylhydrazine in the cold in the proportion of 5 parts sugar, 3 parts water and 5 parts phenylhydrazine deposits within an hour a thick crystalline mass of d-galactose-phenylhydrazone * $C_6H_{12}O_5 : N_2HC_6H_5$. After 24 hours the crystals are filtered off, washed with a little ether and recrystallized from hot alcohol; d-galactose phenylhydrazone forms fine colorless needles, melting at 158° C., easily soluble in hot water and alcohol, but insoluble in ether and chloroform. The 2 per cent aqueous solution is levorotatory † ($[\alpha]_D = -21.6$). The phenylhydrazone reaction may be used for separating d-galactose from d-glucose and other sugars whose hydrazones separate more slowly.

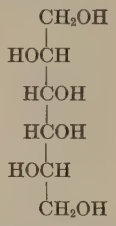
Methylphenylhydrazine and $\beta$-naphthylhydrazine also form with d-galactose insoluble hydrazones which may be employed for purposes of identification.

d-Galactose may be separated from its hydrazones by decomposing the latter with formaldehyde or benzaldehyde according to the usual method.

d-Galactose, or its phenylhydrazone, upon heating with an excess of phenylhydrazine is converted into d-galactose-phenylosazone. ‡ The latter forms fine yellow needles, having the formula $C_6H_{10}O_4(N_2HC_6H_5)_2$ and melting at about 194° to 196° C.; presence of slight impurities may cause, however, marked deviations in the melting point (170° to 190° C.). d-Galactosazone is but slightly soluble in cold water; it is more soluble in hot water and is readily dissolved by hot 60 per cent alcohol.

* Fischer, Ber., **20,** 821. † Jacobi, Ann., **272,** 171. ‡ Fischer, Ber., **17,** 579.

Reduction of d-galactose with sodium amalgam gives the alcohol dulcite, which has the configuration:

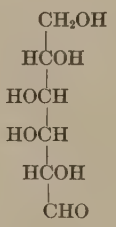


As is evident from its symmetrical structure, dulcite is optically inactive.

*Dulcite* is found in nature in Madagascar manna, *Melampyrum nemorosum* and many other plants. Its melting point is 188° C.

Oxidation of d-galactose with bromine in aqueous solution gives d-galactonic acid, the lactone of which immediately after solution shows a rotation of about $[\alpha]_D = -70$.

**l-Galactose.** —



l-Galactose has been found in nature as a constituent of d, l-galactose among the hydrolytic products of several plant materials; in gum Chagual by Winterstein* and in the Japanese food product, Nori, by Tollens and Oshima.†

l-Galactose has been prepared synthetically‡ by reducing the lactone of l-galactonic acid which is formed together with d-galactonic acid by reduction of mucic acid with sodium amalgam.

*Properties.* — l-Galactose has been obtained as a white crystalline sugar melting at 162° to 163° C., easily soluble in water and 60 per cent alcohol, but only very slightly soluble in absolute alcohol. The sugar is strongly levorotatory, $[\alpha]_D = -74°$ about; strong mutarotation is observed, $[\alpha]_D$ 8 minutes after solution $= -120$.

* Ber., **31**, 1571. † Ber., **34**, 1422.

‡ Fischer and Hertz, Ber., **25**, 1247.

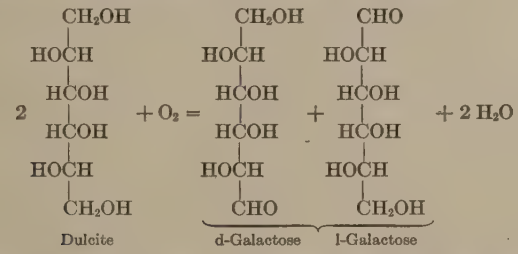
l-Galactose is not fermented by yeast; in this respect the sugar differs from the behavior of d-galactose. This property renders it easy to detect l-galactose in the presence of d-galactose, d-glucose, d-mannose, d-fructose and other fermentable sugars.

*Tests.* — l-Galactose is reduced by sodium amalgam to dulcite and is oxidized by strong nitric acid to mucic acid, the sugar in both these reactions behaving the same as d-galactose; this agreement in behavior, in fact, follows necessarily from the configurations. l-Galactose is oxidized with bromine to l-galactonic acid, whose lactone (not isolated as yet in the pure condition) is dextrorotatory.

l-Galactose forms with phenylhydrazine a difficultly soluble hydrazone melting at 158° to 160° C. and in appearance and solubility very similar to d-galactose-hydrazone. The aqueous solution of l-galactose hydrazone, however, is dextrorotatory, $[\alpha]_D = +21.6$, which distinguishes it from d-galactose hydrazone; l-galactose-phenylosazone resembles in its appearance, melting point and solubility the osazone of d-galactose.

**d, l-Galactose.** — Inactive galactose, as previously noted, has been found in a few cases among the hydrolytic products of certain plant materials.

The sugar has been prepared synthetically * by reducing the lactone of d, l-galactonic acid, which is itself derived by reduction of the lactone of mucic acid; d, l-galactose has also been prepared † by oxidizing dulcite with hydrogen peroxide in presence of iron salts.




*Properties.* — d, l-Galactose, as obtained by Neuberg and Wohlgemuth, ‡ was found to have all the properties of a true racemic substance. The sugar was obtained crystalline, melted at 143° to 144° C., was optically inactive and was fermented only one-half by yeast, the l-galactose remaining unattacked.

* Fischer and Hertz, Ber., **25**, 1247.

† Neuberg and Wohlgemuth, Z. physiol. Chem., **36**, 219.

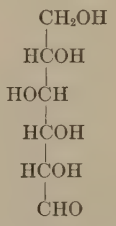
‡ Ibid.

*Tests.* — d, l-Galactose is reduced by sodium amalgam to dulcite and oxidized by strong nitric acid to mucic acid, these reactions being, of course, the same as obtained by each of the component sugars.

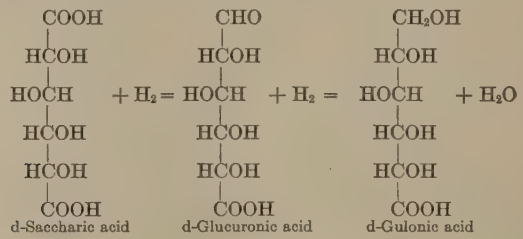
With phenylhydrazine d, l-galactose forms an insoluble hydrazone, which separates rapidly from cold solutions of the sugar and, when purified, consists of colorless crystals melting at 158° to 160° C. The hydrazone is decomposed by heating with formaldehyde or benzaldehyde with liberation of the free sugar, which may then be identified by its optical inactivity, by formation of mucic acid with nitric acid and by leaving a residue of l-galactose after fermentation with yeast.

Oxidation of d, l-galactose with bromine gives d, l-galactonic acid. The latter can be resolved into its components by fractional crystallization of its strychnine salt, the strychnine compound of d-galactonic acid separating as a crystalline deposit while that of l-galactonic acid remains in the mother liquor.

**d-Gulose.** —



d-Gulose has not been identified with certainty in any plant or animal product. The sugar has been prepared synthetically* by reduction of d-saccharic acid, which is converted first to the aldehyde compound d-glucuronic acid, and then to d-gulonic acid.




d-Saccharic acid — d-Glucuronic acid — d-Gulonic acid

The lactone of d-gulonic acid upon reduction with sodium amalgam gives d-gulose.

* Fischer and Piloty, Ber., **24**, 521.

d-Gulose is also formed* by the action of dilute alkalies upon d-sorbose and d-idose.

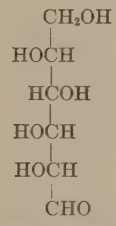
*Properties* — d-Gulose was obtained by van Ekenstein and Blanksma† as white crystals melting at 165° C. and giving a rotation of $[\alpha]_D = + 42.9$. The sugar is not fermentable.

*Tests.* — d-Gulose gives upon reduction with sodium amalgam d-sorbite and upon oxidation with nitric acid d-saccharic acid. In these respects the sugar behaves the same as d-glucose, as follows necessarily from the configuration of the two sugars.

Oxidation of d-gulose with bromine in aqueous solution gives d-gulonic acid, the lactone of which is dextrorotatory ($[\alpha]_D = + 55$ about).

The phenylosazone of d-gulose is identical with that of d-idose and of d-sorbose, these three sugars standing in the same structural relationship to one another as d-mannose, d-glucose and d-fructose.

**l-Gulose.** —



l-Gulose has not been found as yet either free or combined in any natural product. The sugar has been prepared synthetically from l-saccharic acid in the same manner as d-gulose from d-saccharic acid. l-Gulose has also been built up from l-xylose by the addition of hydrocyanic acid and saponification of the nitrile; two acids are formed, as always, in this synthesis, l-idonic and l-gulonic. The lactone of the latter upon reduction gives l-gulose.‡

*Properties.* — l-Gulose has been obtained only as a sweet levorotatory unfermentable sirup ($[\alpha]_D = - 20.4$ about).

*Tests.* — l-Gulose gives upon reduction with sodium amalgam l-sorbite and upon oxidation with nitric acid l-saccharic acid. Oxidation with bromine in aqueous solution gives l-gulonic acid, whose lactone consists of large rhombic hemihedral crystals melting at 181° C. and showing levorotation ($[\alpha]_D = - 55$ about). Oxidation of the lactone

* Lobry de Bruyn and van Ekenstein, Rec. trav. Pays-Bas, **19,** 1.

† Rec. trav. Pays-Bas, **27,** 1.

‡ Fischer and Stahel, Ber., **24,** 528.

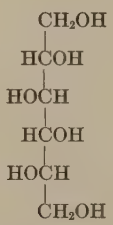
with hydrogen peroxide and basic ferric acetate gives l-xylose, the starting point for the synthesis of l-gulose.

The phenylosazone of l-gulose is identical with that of l-idose and of l-sorbose.

**d, l-Gulose** is obtained * by reducing the lactone of d, l-gulonic acid, which is prepared by mixing equal parts of the lactones of d- and l-gulonic acid. The sugar has been obtained only as a sirup.

The lactone of d, l-gulonic acid crystallizes in prisms melting at 160° C. The crystals as ordinarily obtained from aqueous solution show opposite hemihedry † and the opposite forms when isolated belong to the separate lactones. Such crystals represent, of course, a mixture and not a true racemic combination, which should show only one crystalline form. (See page 785.)

**d-Idose.** —



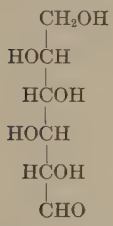
d-Idose has not been found in nature either in the free or combined form. It has been prepared synthetically by Fischer ‡ by reducing the lactone of d-idonic acid, which can be obtained through molecular rearrangement by heating d-gulonic acid with pyridine to 140° C. The sugar is also formed by action of dilute alkalies upon d-gulose and d-sorbose.

*Properties and Tests.* — d-Idose has been obtained only as a clear non-fermentable sirup. Reduction with sodium amalgam gives the alcohol d-idite and oxidation with nitric acid gives d-idosaccharic acid, which has been obtained only as a sirupy mixture of the acid and lactone ($[\alpha]_D$ = over + 100).

d-Idose-phenylosazone is identical with that of d-gulose and d-sorbose.

* Fischer and Curtiss, Ber., **25,** 1025.
† Haushofer, Ber., **24,** 530; **25,** 1027.
‡ Fischer and Fay, Ber., **28,** 1975.

**l-Idose.** —

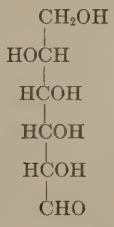


l-Idose has not been discovered as yet in nature. The sugar has been prepared synthetically * by reducing the lactone of l-idonic acid which is prepared from l-xylose by addition of hydrocyanic acid and saponifying the nitrile (see under l-gulose).

*Properties and Tests.* — l-Idose has been obtained only as a colorless non-fermentable sirup ($[\alpha]_D = +7.5$). Reduction with sodium amalgam gives the alcohol l-idite and oxidation with nitric acid gives l-idosaccharic acid, which has been obtained only as a sirupy mixture of acid and lactone ($[\alpha]_D$ = over − 100).

l-Idose-phenylosazone is identical with the phenylosazones of l-gulose and l-sorbose.

**d-Talose.** —



d-Talose has not been discovered as yet in any natural product. The sugar has been prepared synthetically † by reducing the lactone of d-talonic acid, which can be obtained through molecular rearrangement by heating d-galactonic acid with pyridine to 150° C. d-Talose is also formed by action of dilute alkalies upon d-galactose.

*Properties and Tests.* — d-Talose has been obtained only as a sirup ($[\alpha]_D = +13.95$). Reduction with sodium amalgam gives d-talite and oxidation with nitric acid d-talomucic acid. The latter forms microscopic crystals melting at 158° C. and showing dextrorotation, $[\alpha]_D = +29.4$. Oxidation of d-talose with bromine in aqueous solution gives d-talonic

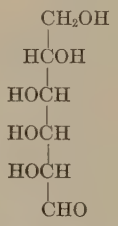
* Fischer and Fay, Ber., **28,** 1975.

† Fischer, Ber., **24,** 3622.

acid, which has been obtained only as a levorotatory sirup consisting of the free acid and lactone.

d-Talose-phenylosazone is the same as that of d-galactose, this identity following from their structural relationship.

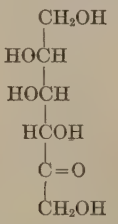
**l-Talose.** —



l-Talose has not been found as yet in nature, nor has its synthesis been accomplished so far as known.

KETOHEXOSES

**D-FRUCTOSE.** — Levulose. Fruit sugar.



**Occurrence.** — d-Fructose is one of the most abundant and widely distributed sugars found in nature. In the free condition it is almost always associated with glucose as a constituent of plant juices, such as the must of fruits, the sap of green leaves and stalks, and the nectar of flowers. Owing to the fact that d-glucose and d-fructose occur so often in very nearly equal amounts, it is supposed that the two sugars are largely formed by the action of inverting enzymes upon sucrose.

The relationship of fructose to glucose and sucrose in the mixed sugars of different plant juices may be seen from the following table:

| | Fructose. | Glucose. | Sucrose. |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| Average of 25 different tropical fruits* | 2.22 | 2.63 | 4.29 |
| Average of green stalks of sugar cane† (12 analyses) | 1.56 | 1.81 | 5.20 |
| Average of green leaves of sugar cane† (2 analyses) | 0.73 | 0.75 | 0.74 |

* Prinsen Geerligs, Chem. Ztg., **21,** 719.
† Browne, Bull., **91,** Louisiana Sugar Experiment Station.

d-Fructose is also widely distributed in nature as a constituent of various anhydride condensation products, such as complex sugars and polysaccharides.

Among the complex sugars, which give d-fructose upon hydrolysis with acids or enzymes, may be mentioned sucrose, raffinose, lupeose, stachyose, secalose and gentianose.

In addition to the complex sugars there are a large number of plant constituents of a gummy character which give d-fructose upon hydrolysis. These substances, which occur mostly as a reserve material in the tubers and root organs of several families of plants, are sometimes called *inuloids* from their chief representative *inulin*. The inuloids have the same general formula $(C_6H_{10}O_5)_n$ with varying amounts of water of hydration and are levorotatory, the values for $[\alpha]_D$ ranging from about $-20$ to $-50$; they are all soluble in hot water, from which solution they are precipitated by absolute alcohol or by the hydroxides of the alkaline earths. The inuloids obtained from different sources are no doubt in very many cases identical, the differences in analysis, specific rotation, melting point, etc., being probably due to accompanying impurities.

**Inulin.** — Inulin occurs very widely distributed as a reserve material in the root organs of the Compositæ and allied families of plants such as the Campanulaceæ, Lobeliaceæ, etc. It was discovered by Rose* in 1804 in the roots of *Inula Helenium* (elecampane), from which plant the compound derives its name. The tuberous roots of the dahlia, dandelion, chicory, Jerusalem artichoke (*Helianthus tuberosus*), arnica and pyrethrum are other examples of plant materials rich in inulin. Owing to its use by plants as a reserve material the percentage of inulin in roots and tubers is subject to wide fluctuations, being usually least in spring and greatest in autumn. Dandelion roots, for example, were found by Dragendorff† to contain 1.74 per cent inulin in March and 24 per cent in October. The dahlia, chicory, pyrethrum and Jerusalem artichoke may contain over 50 per cent inulin in the dry substance of the roots.

*Preparation of Inulin.* — Inulin may be prepared according to Kiliani‡ by reducing to a fine pulp the ripe tubers (taken in autumn) of the dahlia, chicory, etc., boiling the material with water in presence of calcium carbonate and filtering. The filtrate is then frozen in a freezing mixture and after thawing out the precipitated inulin filtered off;

* Gehlen's Neues allgem. J. Chem., **3**, 217.
† "Monographie des Inulins" (1870).
‡ Ann., **205**, 147.

the raw product thus obtained is redissolved in hot water and again frozen out. After repeating the purification in this way for several times the final product is washed with 93 per cent alcohol, then with a little ether and afterwards carefully dried in a water oven.

Tanret* recommends a preliminary clarification of the hot root extracts with lead subacetate; after filtering, the solution is freed from lead by sulphuric acid and then the inulin precipitated by adding a concentrated solution of barium hydroxide and heating. The precipitate is washed with cold barium hydroxide solution and then decomposed in aqueous suspension with carbon dioxide; the solution after heating is filtered from barium carbonate, and the inulin precipitated from the filtrate by means of strong alcohol.

Fig. 194. — Sphere-crystals of inulin (precipitated from cells of the dahlia by means of alcohol).

*Properties of Inulin.* — Inulin, as generally prepared, has a composition of the formula $6(C_6H_{10}O_5) + H_2O$, i.e., $C_{36}H_{62}O_{31}$ or a multiple thereof. Brown and Morris† give the formula $C_{72}H_{124}O_{62}$ and Tanret $C_{180}H_{310}O_{155}$. Inulin consists of a white hygroscopic substance, very easily soluble in hot water, in which it may form supersaturated solutions. It is insoluble in absolute alcohol. Its aqueous solution does not reduce Fehling's solution, and gives no color reaction with iodine (distinction from soluble starch, plant glycogen, and dextrin). Inulin in aqueous solution is levorotatory, $[\alpha]_D = -38$ (about), the values as determined by different authorities for different preparations ranging from $-36$ to $-40$.

* Bull. soc. chim. [3], **9**, 201. † Chem. News, **59**, 296.

Inulin is rapidly hydrolyzed (15 to 20 minutes) to d-fructose upon heating with dilute acids. Hydrolysis may also be effected by heating with water alone under pressure at 110° to 120° C. A special enzyme *inulase* which is found in the germinating tubers of the Jerusalem artichoke, and other inulin-containing plants, also hydrolyzes inulin to d-fructose; other enzymes such as diastase, invertase, emulsin, etc., are without action. Inulin is not fermented by yeast.

Inulin can usually be detected in plant tissues by placing thin sections of the tubers, etc., in strong alcohol or glycerol and then examining the preparation under the microscope. The inulin will be precipitated within the cells as sphere-crystals marked with radial fissures (Fig. 194).

In addition to inulin Tanret,* by the fractional precipitation of the extract from Jerusalem artichokes with barium hydroxide, has separated the following closely allied compounds:

| | $[\alpha]_D$. |
|---|---|
| Pseudoinulin | − 32.2 |
| Inulenin | − 29.6 |
| Helianthenin | − 23.5 |
| Synanthrin | − 17.0. |

Among other inuloid substances† found in different plant materials may be mentioned the following:

| Compound. | Source. | $[\alpha]_D$. |
|---|---|---|
| Levosin | Unripe grain or cereals | −36 |
| Phlein | Roots of *Phleum pratense* (timothy grass) | −48 |
| Irisin | Roots of *Iris pseudacorus* | −51.5 |
| Graminin | Roots of different grasses | −38 to −44 |
| Triticin | Roots of *Triticum repens* | −36 to −50 |
| Scillin or Sinistrin | Bulbs of *Urginea Scilla* and other plants | −34 to −48 |

*Levan and Levulan.* — d-Fructose also occurs as an anhydride condensation product in many gums of bacterial formation, such as levan ($[\alpha]_D = -40$), which was found by Greig-Smith and Steel‡ to be produced by the organism *Bacillus levaniformans* in the raw products of cane sugar factories. A similar gum levulan ($[\alpha]_D = -221$) was found by Lippmann§ in beet molasses.

d-Fructose is also found in animal products although much less

* Bull. soc. chim. [3], **9**, 202, 623.

† For a fuller description of the many inuloid substances see Lippmann's "Chemie der Zuckerarten," 800.

‡ The Sugar Cane, II, **4**, 481; **5**, 448.

§ Ber., **14**, 1509; **25**, 3216.

commonly than d-glucose, and frequently only as a result of disease or other abnormal condition. It occurs at times in normal urine, as after eating excessive amounts of sweet meats or drinking sweet wines, champagnes, etc. d-Fructose is also found in the urine of certain diabetic patients; such urine even when rich in fructose may show but little levorotation owing to the counter effect of the rotation of d-glucose. Levorotation in urine may also be produced by glucuronic acid complexes (p. 375), so that an optical examination of urine without confirmatory tests is not always to be relied upon.

*Honey and Floral Nectar.* — The occurrence of d-fructose in honey has already been referred to. The average amount of fructose, glucose and sucrose in honey according to different authorities is given as follows:

| | Fructose. | Glucose. | Sucrose. |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| 138 European honeys (König*) | 38.65 | 34.48 | 1.76 |
| 92 American honeys (Browne†) | 40.50 | 34.02 | 1.90 |

* König's "Chem. Nahrungs- und Genussmittel," 3d ed., I, 766.
† Bull. 110, U. S. Bur. Chem., p. 38.

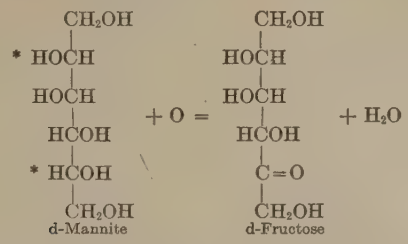
It is seen from the above that fructose occurs in honey in slightly greater excess than glucose. A part of the fructose and glucose of honey is due to the inversion of sucrose gathered by bees from floral nectar and other sources. The sucrose in Sainfoin nectar according to Bonnier* constitutes 57.2 per cent of the total sugars and in Sainfoin honey only 8.2 per cent, which shows that over 85 per cent of the sucrose in the nectar was inverted by the bees. This inversion takes place while the nectar is in the honey sac of the bee, and also no doubt during evaporation and storage of the nectar in the comb; the inverting agent is probably an enzyme secreted by the bee and the process is found to continue even after the honey has been strained.

In certain honeys, as those gathered by bees from the flowers of the Eucalyptus and Tupelo, the fructose is found in very large excess, a circumstance which is probably due to the preponderance of fructose in the nectar of these flowers.

**Synthesis of d-Fructose.** — d-Fructose has been made synthetically in a large number of ways. Fischer's method of synthesis from d-glucose by reducing its osone has been described (p. 355); also the method of Lobry de Bruyn and van Ekenstein by which d-glucose and d-mannose undergo partial rearrangement to d-fructose upon warming in

* "Sources of Honey," Sci. Amer. Suppl., Aug. 10, 1907, p. 92.

dilute alkaline solution. The synthesis can also be accomplished biologically from d-mannite, which is oxidized by the sorbose bacterium almost quantitatively to d-fructose.*




The oxidation of d-mannite by the sorbose bacterium may take place (p. 771) in the second or fifth position (marked by a *) with formation of d-fructose in either case.

**Preparation of d-Fructose.** — d-Fructose is most easily prepared by hydrolyzing some one of its condensation products. For this purpose sucrose and inulin are the substances generally chosen. The sugar on account of its extreme solubility is very difficult to crystallize.

*Preparation of d-Fructose from Sucrose.* — In the preparation† from sucrose a solution of invert sugar is prepared by inverting a 10 per cent solution of sucrose at 60° C., using for every 100 gms. of sucrose 2 c.c. of concentrated hydrochloric acid. The solution after cooling to about − 5° C. is treated for each 100 c.c. with 6 gms. of fresh finely pulverized pure calcium hydroxide. After stirring the solution vigorously for 2 to 3 minutes, the liquid is filtered rapidly using a cooling funnel; the filtrate which should be kept cold soon deposits fine needles of calcium fructosate. The latter after standing 24 hours is filtered off, using a centrifuge or Buchner funnel, washed with ice water and then after suspending in water at 20° C. carefully decomposed with the exact amount of oxalic acid. Any excess of the latter may be removed by addition of a little of the calcium fructosate kept back as a reserve. The filtrate from the calcium oxalate is then evaporated at low temperature in a vacuum to a sirup. The latter after priming with a crystal of fructose from a previous preparation and setting aside in a cool place over concentrated sulphuric acid will usually crystallize within a few days. Crystallization may also be effected by dissolving as much as possible of the concentrated sirup in warm absolute

* Brown, J. Chem. Soc., **49.** 172.

† Modification of the original method of Dubrunfaut, Compt. rend., **25,** 307; **69,** 1366.

alcohol, and then pouring off, when cold, the soluble portion from the sirupy residue; the alcoholic solution upon standing will soon deposit crystals of pure d-fructose.

*Preparation of d-Fructose from Inulin.* — d-Fructose is most easily prepared from inulin. For this purpose* 100 gms. of inulin and 250 c.c. of water are heated with a little hydrochloric acid for 30 minutes in a boiling water bath. The quantity of hydrochloric acid used for hydrolyzing depends upon the ash content of the inulin; for 100 gms. inulin of 1 per cent ash content 0.5 gm. HCl is taken, for 0.2 per cent ash 0.1 gm. HCl and if the inulin is ash free 0.01 gm. HCl. After hydrolysis the free acid is neutralized with an excess of calcium carbonate and the solution filtered. The filtrate is evaporated in a vacuum at low temperature to a thin sirup, which is then set aside in a vacuum desiccator over concentrated sulphuric acid. After standing some days the thick sirup is warmed with absolute alcohol and after thorough agitation allowed to stand 24 hours. The clear alcoholic solution is then poured off, primed with a few crystals of fructose and set aside in a cool place. Crystallization is usually complete in 3 days. The sugar is obtained perfectly white by recrystallizing from hot absolute alcohol using bone black.

**Properties of d-Fructose.** — d-Fructose crystallizes from absolute ethyl or methyl alcohol as the anhydride $C_6H_{12}O_6$ in fine colorless needles melting at 95° to 105° C. A crystalline hydrate of the formula $(C_6H_{12}O_6)_2 + H_2O$ has also been obtained.

d-Fructose is exceedingly soluble in cold water, but only very slightly soluble in cold absolute alcohol. It is easily soluble in hot absolute ethyl and methyl alcohol. Unlike most sugars d-fructose is soluble to a considerable extent in mixtures of alcohol and ether.

d-Fructose is very strongly levorotatory, $[\alpha]_D^{20} = -92$ although changes in temperature and concentration may produce considerable variations from this figure as shown on page 181.

Diluting a concentrated fructose solution with water causes a lowering of the specific rotation, and about 30 minutes are necessary for the reading to become constant.

d-Fructose exhibits mutarotation, the value for $[\alpha]_D$ immediately after solution being about $-106$. The change to constant rotation proceeds much faster than with other mutarotating sugars, and is usually completed within an hour.

The effect of acid in increasing the levorotation of d-fructose has been referred to.

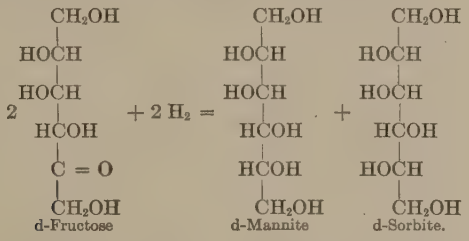
* Ost. Z. analyt. Chem., **29.** 648.

**Fermentation of d-Fructose.** — d-Fructose is fermented in the same manner as d-glucose by various yeasts, moulds and bacteria. Yeast produces about the same yield of alcohol and carbon dioxide from d-fructose as from d-glucose, but the fermentation in its first stages proceeds more rapidly with glucose. The alcoholic fermentation of fructose by means of the enzyme zymase has been accomplished by Buchner in the same manner as for glucose.

d-Fructose undergoes the lactic and butyric fermentations with the same readiness as d-glucose.

In certain anaërobic fermentations where free hydrogen is evolved d-fructose is reduced to d-mannite, the reaction being of the same character as that obtained with sodium amalgam and other reducing agents. The formation of mannite by microörganisms in fructose-containing solutions is often termed a mannitic fermentation.

**Tests for d-Fructose.** — d-Fructose upon reduction with sodium amalgam yields equal parts of d-mannite and d-sorbite.



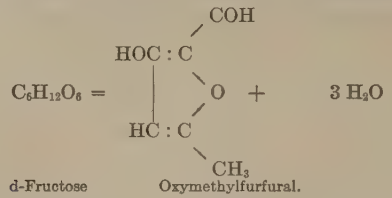

The above reaction, by which two alcohols are formed, is characteristic of all ketoses (see under d-erythrulose, page 543).

Oxidation of d-fructose by means of bromine water proceeds less rapidly than with the aldehyde sugars and this property has been utilized as a means of identification (p. 363). By prolonged action of bromine water extending over several weeks, d-fructose is oxidized* to a mixture of formic, oxalic, glycollic and d-erythronic acids. Oxidation of d-fructose with nitric acid gives a mixture of formic, oxalic, tartaric and glycollic acids.

d-Fructose gives a number of brilliant color reactions which are more typical, however, of the ketose sugars as a class, than of d-fructose alone. The intense red color reaction of Seliwanoff, obtained upon heating fructose solutions with resorcin and strong hydrochloric acid, has already been described.

* Herzfeld, Ann., **244**, 291.

If solutions of d-fructose are heated to a high temperature the sugar is partly decomposed with formation of *oxymethylfurfural.*




*Tests for Artificial Invert Sugar.* — The oxymethylfurfural formed in the previous reaction is easily detected by its coloring aniline acetate red or by its forming brilliant colorations with phloroglucin, resorcin and other phenols. This property has been made use of for detecting artificial invert sugar in honey, and other food products. Artificial invert sugar is made commercially by heating concentrated sucrose solutions with a small amount of tartaric or other acid (about 0.1 per cent of weight of sucrose) to 110° to 120° C., at which temperature perceptible amounts of oxymethylfurfural are formed.

In making the test for oxymethylfurfural Fiehe* rubs up the product (honey, etc.) with ether and filters the ethereal solution into a small porcelain dish. After evaporating the ether, the residue is heated with a 1 per cent solution of resorcin in concentrated hydrochloric acid. In presence of artificial invert sugar a red color develops which soon changes to a reddish brown.

A more rapid but less sensitive reaction for artificial invert sugar is obtained with aniline acetate.† The reagent, which should be freshly prepared before using, is made by shaking up 5 c.c. of chemically pure aniline with 5 c.c. of water and adding sufficient glacial acetic acid (2 c.c.) to just clear the emulsion. In making the test 5 c.c. of a concentrated solution of the honey, etc., are treated in a test tube with 1 to 2 c.c. of the aniline reagent. The latter is allowed to flow down the walls of the tube so as to form a layer upon the surface of the solution underneath. If a red ring forms beneath the aniline solution, when the tube is gently agitated, oxymethylfurfural is present.

It should be borne in mind that honeys or other fructose-containing products which have been cooked or boiled also give the reaction for oxymethylfurfural.

The brilliant red coloration obtained upon heating d-fructose or sucrose with concentrated hydrochloric acid and sesame oil is probably

* Z. Nahr. Genussmittel, **16,** 75.

† Browne, Bull., 110, U. S. Bur. Chem., p. 68.

due to a condensation product between oxymethylfurfural and some constituent of the oil.

Hydrobromic acid reacts with fructose in ether solution to form *bromomethylfurfural* $CH_2Br \cdot C_4H_2O \cdot CHO$, which colors the solution a deep reddish purple and crystallizes in gold colored prisms. (Reaction of Fenton and Gostling.*) This reaction is also given by other sugars and carbohydrates, but is most pronounced with those which contain a fructose group.

*Reducing Reactions of d-Fructose.* — d-Fructose is more sensitive in reducing power than most other sugars and this property has been utilized as a means of identification.

Pinoff † recommends for the above purpose a 4 per cent solution of ammonium molybdate; 10 c.c. of the latter diluted with 10 c.c. of water containing 0.2 c.c. of glacial acetic acid gives upon heating with 0.1 gm. d-fructose in a water bath at 95° to 98° C. a bright blue coloration; solutions of other sugars under these conditions remain colorless. Any free mineral acid must be neutralized before conducting the experiment, otherwise other sugars may give the reaction.

Pieraerts ‡ recommends for detecting fructose a solution of copper hydroxide in potassium carbonate or in alkaline amino-acetic acid (glycocoll). The latter reagent is prepared by dissolving 12 gms. of glycocoll in water; 6 gms. of copper hydroxide are then added gradually and when solution is complete the hot liquid is cooled to 60° C.; 50 gms. of potassium carbonate are then added and the solution made up to 1 liter. In testing for fructose the product to be examined is dissolved in cold water, clarified if necessary with a little lead acetate, the filtrate freed from excess of lead by means of sodium sulphate and the clear solution diluted to about 5 per cent reducing sugar. Upon heating with the alkaline glycocoll-copper solution to 30° C. reduction will take place within an hour if fructose is present; reduction is also obtained at ordinary temperature after 12 hours' standing. Other sugars are said not to show reduction under these conditions.

*Methylphenylosazone Reaction of d-Fructose.* — d-Fructose forms a large number of hydrazones and osazones with phenylhydrazine and its substituted derivatives. For purposes of separation and identification the osazone reaction with methylphenylhydrazine is stated by Neuberg § to be of great value. d-Fructose-methylphenylosazone is

* J. Chem. Soc., **73,** 556; **75,** 423; **79,** 361. † Ber., **38,** 3317.

‡ Belg. Ann. de Pharmacie, March and April, 1908. Bull. assoc. chim. sucr. dist., **25,** 830.

§ Ber., **35,** 959. Z. physiol. Chem., **36,** 227.

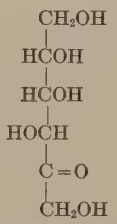
obtained upon heating fructose solutions with methylphenylhydrazine in alcoholic acetic acid for a few minutes and then setting aside in a cool place. Crystallization is almost complete within a few hours. The compound consists of yellowish crystals having the composition $C_{20}H_{26}N_4O_4$, and melting between 158° and 160° C.; it is only slightly soluble in water, cold alcohol and ether, but is easily soluble in hot alcohol, acetone and chloroform.

Glucose, mannose, galactose and other aldose sugars do not form osazones with methylphenylhydrazine owing to the fact that the $-CHOH$ group adjoining the terminal $-CHO$ radicle is prevented in some way from reacting with substituted hydrazines. In the case of fructose and other ketoses, where the reacting $-CH_2OH$ group occupies the end position, the freedom of osazone formation is not impeded.

d-Fructose-phenylosazone is identical with that of d-glucose and d-mannose. The formation of osazone is more complete, however, with d-fructose than with its aldose isomers (see page 350).

d-Fructose reacts with alkalies similarly to d-glucose.

**l-Fructose.** —



l-Fructose has not been found as yet in nature. The sugar has been prepared synthetically* by reduction of l-glucosone (obtained from l-glucose-osazone), in the same manner as d-fructose is prepared from d-glucose-osazone; l-fructose can also be prepared from d, l-fructose by fermenting away the d-component with yeast.

l-Fructose has been obtained only as a dextrorotatory unfermentable sirup. The sugar has not been separated in a pure crystallized form, so that other knowledge of its chemical and physical properties is lacking.

**d, l-Fructose.** — Inactive, or racemic, fructose has not been found in nature. The sugar has been prepared synthetically, however, by a number of methods and some of these have a special interest in that the sugar was built up from simple organic compounds not belonging

* Fischer, Ber. **23,** 373, 2618, 3889; **24,** 2683.

to the sugars. The best known example of such a method is the classic synthesis of Fischer and Tafel * by which acrolein dibromide in contact with barium hydroxide is condensed to form α-acrose and other hexose sugars.

$$\underset{\text{Acroleindibromide}}{2\,C_3H_4Br_2O} + 2\,Ba(OH)_2 = 2\,BaBr_2 + \underset{\alpha\text{-Acrose.}}{C_6H_{12}O_6}.$$

The above reaction is carried out in ice-water. The solution, after precipitating barium, is evaporated and treated with phenylhydrazine. Two osazones are formed, one insoluble in ether (α-acrose-osazone) and the other soluble in ether (β-acrose-osazone). The α-acrose-osazone is found to be identical with that of d, l-glucose, which is also the same as that of d, l-mannose, or d, l-fructose.

α-Acrose-osazone upon treatment with zinc dust and acetic acid is reduced to α-acrose-amine, and the latter upon treatment with nitrous acid is converted to d, l-fructose.

$$2\ \underset{\alpha\text{-Acrose-amine}}{\begin{array}{l}CH_2OH\\|\\(CHOH)_3\\|\\C{=}O\\|\\H_2C{-}NH_2\end{array}} + 2\,HNO_2 = 2\ \underset{\text{d,l-Fructose.}}{\begin{array}{l}CH_2OH\\|\\(CHOH)_3\\|\\C{=}O\\|\\CH_2OH\end{array}} + 2\,N_2 + 2\,H_2O$$

*Properties.* — d, l-Fructose has been obtained only as a sweet colorless optically inactive sirup, easily soluble in water and alcohol, but insoluble in ether. It reduces Fehling's solution and gives the other common reactions of a reducing sugar. It is fermented only one-half with yeast, the l-fructose remaining in an unaltered condition.

Reduction of d, l-fructose with sodium amalgam gives d, l-mannite, the oxidation of which to d, l-mannonic acid has been mentioned in the synthesis of d-mannose and d-glucose.

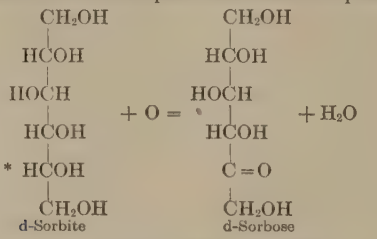
**D-SORBOSE.** — d-Sorbinose.

$$\begin{array}{l}CH_2OH\\|\\HCOH\\|\\HOCH\\|\\HCOH\\|\\C{=}O\\|\\CH_2OH\end{array}$$

**Occurrence.** — Although d-sorbose has not been found free in the natural juices of plants this sugar has been discovered in large quan-

* Ber., **20,** 2566; **22,** 97.

tities in the fermented juice of sorb-apples (the fruit of the service-tree, *Sorbus domestica*) and other fruits of the rosaceous family. The sugar was discovered first by Pelouze,* but Boussingault† was the first to show that the sugar was formed by an oxidizing fermentation of the hexite alcohol d-sorbite which is found in sorb-apples, mountain-ash berries and other fruits. One of the principal organisms concerned in this fermentation is the so-called sorbose bacterium (*Bacterium xylinum*), the action of which upon d-sorbite is represented as follows:




The oxidation takes place only in the position marked with a *, as explained on page 771.

d-Sorbose is formed synthetically in small amounts by the action of dilute alkalies upon d-gulose and d-idose.

**Preparation of d-Sorbose.** — d-Sorbose is prepared according to Bertrand's ‡ method by evaporating the juice of sorb-apples to a specific gravity of 1.05 to 1.06 and then removing all fermentable sugars by fermentation with yeast. When the alcoholic fermentation is completed the clear solution is poured into shallow dishes, inoculated with a strong pure culture of *Bacterium xylinum* and allowed to stand at 30° C. until the reducing power of the solution has reached its maximum. The liquid is then clarified with lead subacetate, the excess of lead removed from the filtrate with the exact amount of sulphuric acid and the filtrate, which must be perfectly neutral, evaporated in a vacuum to a sirup. The latter is purified in the usual way by strong alcohol and set aside for crystallization. The yield of d-sorbose is about 80 per cent of the d-sorbite originally present.

Instead of using sorb-apple juice a solution of d-sorbite in presence of yeast extract, asparagine, peptone and mineral nutrients may be used for fermentation. By this method Lobry de Bruyn and van Ekenstein § obtained a yield of about 30 per cent.

**Properties.** — d-Sorbose has been obtained in the form of colorless rhombic crystals melting at 154° C. The sugar is very sweet, easily

* Compt. rend., **34**, 377.
† Compt. rend., **74**, 939.
‡ Compt. rend., **122**, 900.
§ Rec. trav. Pays-Bas, **19**, 3

soluble in water, but difficultly soluble in alcohol. d-Sorbose is levorotatory, $[\alpha]_D = -42.5$, this value being slightly influenced by changes in temperature and concentration as shown on page 181.

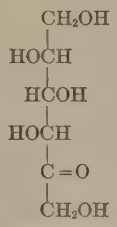
d-Sorbose is not fermented by any of the ordinary varieties of yeast. The sugar is also very resistant to the attacks of moulds and bacteria; certain lactic acid organisms, however, were found by Berthelot * to produce lactic and butyric acid.

**Tests.** — d-Sorbose is reduced by sodium amalgam to the alcohols d-sorbite and d-idite. Oxidation with nitric acid produces d-tartaric, oxalic and other acids.

d-Sorbose reduces Fehling's solution and gives the characteristic color reaction of the ketoses with resorcin and hydrochloric acid.

Upon heating with 3 parts of phenylhydrazine chloride and 5 parts of sodium acetate d-sorbose gives an osazone, $C_{18}H_{22}N_4O_4$, which is identical with that of d-gulose and d-idose. The osazone consists of fine yellow needles melting at 164° C.

**l-Sorbose.** — l-Sorbinose.



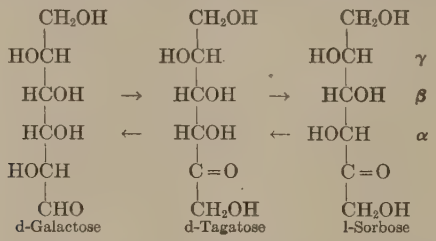
*Synthesis.* — l-Sorbose has not been found in nature either free or in the combined form. The sugar has been prepared synthetically by Lobry de Bruyn and van Ekenstein † by warming d-galactose in 20 per cent aqueous solution with not more than 3 per cent potassium hydroxide for 3 hours at 70° C. The solution, which has acquired a weak acid reaction, is cooled and the unchanged galactose allowed to crystallize. The mother liquor is then evaporated, and extracted with methyl alcohol and acetone. The residue is then fermented to remove the rest of the galactose and the solution evaporated to a sirup, from which the l-sorbose crystallizes after long standing. The yield is 6 to 8 per cent of the d-galactose taken.

The above reaction by which l-sorbose is formed belongs to a class of secondary rearrangements which are peculiar to many of the sugars. As d-glucose upon warming with dilute alkalies undergoes partial

* Ann. chim. phys. [3], **50**, 350.

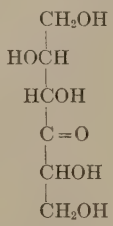
† Rec. trav. Pays-Bas, **16**, 262; **19**, 1.

rearrangement into d-mannose and the ketone sugar d-fructose, so d-galactose is transformed into d-talose and the ketone sugar d-tagatose. The ketone sugars which are formed in these reactions seem, however, on prolonged warming with alkalies to be partially transformed into isomeric ketoses. The reaction between d-galactose, d-tagatose and l-sorbose would be represented as follows:




The rearrangement between d-tagatose and l-sorbose involves the transposition of the H and OH groups in the $\alpha$ position to the CO group, a change somewhat analogous to that noted in the rearrangements between the aldose sugars (as d-glucose to d-mannose) and between the sugar acids (as d-gluconic to d-mannonic) where the transposition of the H and OH groups occurs in the $\alpha$ position to the CHO and COOH groups respectively.

The rearrangement between d-tagatose and l-sorbose is also accompanied by the formation of other ketoses such as galtose in which the CO group may perhaps take the following position:




It can readily be seen that the possible number of isomeric ketoses, which successive rearrangements of this kind may bring about, is large.

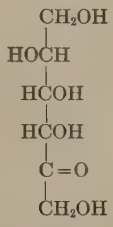
*Properties of l-Sorbose.* — l-Sorbose, as obtained by the method of Lobry de Bruyn and van Ekenstein, consists of colorless rhombic crystals melting at 154° to 156° C. The sugar is dextrorotatory, $[\alpha]_D = +42.3$ without showing perceptible mutarotation. Changes in temperature seem to have no marked influence upon the rotation. The sugar is not fermented with yeast.

*Tests.* — l-Sorbose is reduced by sodium amalgam to the hexite alcohols l-sorbite and l-idite. The sugar reduces Fehling's solution somewhat stronger than d-galactose and gives the characteristic color reaction of ketoses with resorcin and hydrochloric acid.

The phenylosazone is identical with that of l-idose and l-gulose.

**d, l-Sorbose.** — This sugar was prepared by Lobry de Bruyn and van Ekenstein* by evaporating a solution containing equal parts of d- and l-sorbose. The sugar was obtained in white crystals melting at 154° C. A study by Adriani † of its solubility as compared with that of its two components showed that the crystals were a true racemic combination and not a simple mixture.

**d-Tagatose.** —



*Synthesis.* — d-Tagatose has not been found as yet in nature either free or in any combined form. The sugar has been prepared synthetically by Lobry de Bruyn and van Ekenstein‡ by the action of dilute alkalies upon d-galactose as described under l-sorbose. The mother liquor after crystallization of l-sorbose yields upon evaporation a mixture of crystals consisting of l-sorbose and d-tagatose. The mixed crystals are dissolved in 5 parts of absolute methyl alcohol and 2 parts aniline, from which the l-sorbose crystallizes at once and the d-tagatose after evaporating the mother liquor. The sugar is purified by recrystallization.

*Properties.* — d-Tagatose consists of white crystals melting at 124° C. The sugar has a sweet taste and is easily soluble in water, but difficultly soluble in alcohol. The aqueous solution is very weakly dextrorotatory, $[\alpha]_D^{22} = +1$; at higher temperatures the sugar is levorotatory, $[\alpha]_D^{60} = -2.6$. d-Tagatose is not fermented by yeast.

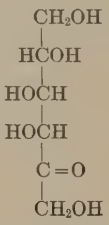
*Tests.* — d-Tagatose gives upon reduction with sodium amalgam the hexite alcohols dulcite and d-talite. Oxidation with nitric acid

* Rec. trav. Pays-Bas, **19,** 1.
† Rec. trav. Pays-Bas, **19,** 185.
‡ Rec. trav. Pays-Bas, **16,** 62, 282; **18,** 72.

causes a disintegration of the carbon chain with formation of l-tartaric, oxalic and other acids. The sugar reduces Fehling's solution somewhat stronger than d-galactose and gives the characteristic color reaction of ketoses with resorcin and hydrochloric acid.

The phenylosazone is identical with that of d-galactose and d-talose.

**l-Tagatose.** —



l-Tagatose has not been found as yet in nature. The sugar is formed by molecular rearrangement according to Lobry de Bruyn and van Ekenstein* by the action of dilute alkalies upon d-sorbose, the transformation being the same as that between l-sorbose and d-tagatose.

The sugar has not been isolated as yet in the crystalline form and its properties are therefore unknown. Its phenylosazone is identical with that of l-galactose and l-talose.

**d, l-Tagatose.** — Racemic, or inactive, tagatose has not been found in nature, nor has the sugar up to the present been prepared synthetically. An inactive ketose sugar† has been detected among the oxidation products of dulcite obtained by action of lead peroxide, and this sugar in all probability consists in part at least of d, l-tagatose.

### KETOHEXOSES OF UNKNOWN STRUCTURE

**Galtose.**‡ — This ketohexose has already been referred to under l-sorbose as being formed by the action of alkalies upon d-galactose through secondary rearrangement. The sugar remains in the mother liquors after crystallization of the l-sorbose and d-tagatose.

Galtose has been obtained only as a sweet sirup. Its aqueous solutions have but little rotatory power and are not fermentable. Galtose reduces Fehling's solution only about half as strong as d-galactose. Distillation with hydrochloric acid gives 4 to 5 per cent of furfural.

* Rec. trav. Pays-Bas, **16,** 62, 282; **18,** 72.

† Neuberg, Ber., **35,** 2629.

‡ Lobry de Bruyn and van Ekenstein, Rec. trav. Pays-Bas, **16,** 257, 262.

Galtose-phenylosazone, $C_{18}H_{22}N_4O_4$, forms yellow crystals melting at 182° C.

**Glutose.*** — This ketohexose is formed by secondary rearrangement through the action of dilute alkalies upon d-glucose, d-mannose and d-fructose. The best yields are obtained by heating a 20 per cent solution of d-glucose or d-fructose with 10 per cent of pure moist lead hydroxide for 3 hours at 70° C. The lead is then precipitated, the mixture of sugars fermented with yeast, when the glutose remains behind. The yield of glutose by this method is about 20 per cent from d-glucose and 40 per cent from d-fructose.

*Occurrence of Glutose in Cane Molasses.* — While glutose does not occur in nature its presence can always be looked for in commercial products where d-glucose and d-fructose have been subjected to the action of alkalies. It has been found in sugar-cane molasses† in amounts varying from 1 to 5 per cent as a result of the action of the lime used in clarification upon the invert sugar of the juice. Glutose, not being fermentable, is found as a constituent of the vinasse from molasses distilleries. In the valuation of molasses for distilleries the amount of glutose and other non-fermentable reducing sugars should be determined by a carefully conducted fermentation test.

*Properties.* — Glutose has not been obtained in the crystalline form, so nothing is known of its physical properties. It reduces Fehling's solution about half as strong as d-glucose and gives the characteristic color reaction of ketoses with resorcin and hydrochloric acid. Aqueous solutions of glutose show no perceptible optical activity.

**Pseudofructose.** — This ketohexose has also been detected among the products obtained by action of dilute alkalies upon d-glucose or d-fructose. The sugar was obtained by Lobry de Bruyn and van Ekenstein‡ as a levorotatory sirup ($[\alpha]_D = -40$ about) but has not been isolated in the pure condition.

**Formose.** — The condensation of formaldehyde in presence of alkalies to a sweet sugar-like substance was first observed by Butlerow,§ who gave the compound the name methylenitan. A similar condensation product was afterwards prepared by Loew‖ who gave it the name

* Lobry de Bruyn and van Ekenstein, Rec. trav. Pays-Bas, **16,** 62, 282.
† Pellet, Bull. assoc. chim. sucr. dist., **16,** 1181; **19,** 834.
‡ Rec. trav. Pays-Bas, **16,** 162.
§ Compt. rend., **53,** 143.
‖ J. prakt. Chem. [2], **33,** 321.

formose. Methylenitan and formose are according to Fischer identical in nature, although this has been disputed by Loew and Tollens. To prepare formose a 4 per cent solution of formaldehyde is shaken with an excess of milk of lime for half an hour and then filtered; the alkaline filtrate is allowed to stand for 5 to 6 days at room temperature when the condensation is complete. The solution is then neutralized with oxalic acid, filtered and evaporated to a thin sirup; the latter is purified from lime salts and other impurities by means of strong alcohol; the alcoholic solution upon evaporation gives a residue which consists mostly of formose.

*Properties.* — Formose has been obtained only as a yellowish intensely sweet sirup; it is optically inactive and strongly reducing. It is not fermented by yeast, although certain organisms decompose the sugar with formation of lactic acid. The sugar, from the analysis of its osazone, belongs to the hexoses, and from its color reaction with resorcin and hydrochloric acid is a ketose. The configuration of formose has not as yet been determined and it is still a question whether formose is not a mixture* of sugars rather than a single substance.

Formose-phenylosazone, $C_{18}H_{22}N_4O_4$, was obtained by Fischer† as fine yellow needles melting at 144° C.

**β-Formose**‡ and **morfose**§ are two other sugars which Loew has obtained from formaldehyde by varying the temperature and other conditions of condensation. The sugars have been obtained only as impure sirups; the existence of these sugars has been strongly questioned.

**Lycerose**‖ was obtained by Loew as an impure sirup through condensation of glycerose with calcium hydroxide at 75° C. In all probability lycerose is a mixture of several condensation products.

### NATURAL HEXOSES OF UNCERTAIN CHARACTER

A large number of hexose sugars of unknown configuration have been reported at various times in the literature. The existence of these in nearly all cases requires confirmation. It is possible that some of the sugars in the following list belong to some one of the hexoses

* According to Nef (Ann., **376,** 1) synthetic formose is probably a mixture of all possible aldo- and keto-tetroses, -pentoses and -hexoses, in the equilibrium between which some 116 different substances take part.

† Ber., **21,** 988.

‡ J. prakt. Chem. [2], **34,** 51.

§ Chem. Ztg., **23,** 542.

‖ Chem. Ztg., **23,** 542.

previously described, the variations noted in specific rotation and other properties being due to impurities.

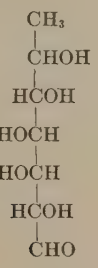
| Sugar.* | Source of sugar. | Properties. |
|---|---|---|
| Convallamarin sugar. | Glucoside from lily of the valley. | |
| Hederose | Glucoside from ivy | Crystals, $[\alpha]_D = +102.7$ |
| Locaose | Glucoside from rhamnus bark | Crystals, $[\alpha]_D = 0$ |
| Saporubrose | Glucoside from soapwort | $[\alpha]_D = +23.7$ |
| Scammonose | Glucoside from scammony | $[\alpha]_D = +17.8$ |
| Skimminose | Glucoside from skimmia | Crystals, $[\alpha]_D = +24.5$ |
| Solanose | Glucoside from potato | |
| Chondroglucose | Hydrolysis of cartilage | Crystals, $[\alpha]_j = -45.8$ |
| Mucose | Hydrolysis of mucin | |

* For a fuller account of these and other sugars of uncertain character the chemist is referred to the long list in Lippmann's "Chemie der Zuckerarten" (1904), 975.

## Methylhexoses

$CH_3 \cdot C_6H_{11}O_6$

**α-Rhamnohexose.** —



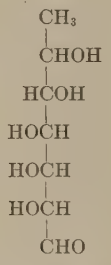
By addition of hydrocyanic acid to rhamnose and saponification of the nitrile with barium hydroxide, two isomeric rhamnohexonic acids should result. In this reaction, however, one isomer is always formed with more readiness than the other and so in the case of rhamnose α-rhamnohexonic acid is produced in much greater amount. The lactone of this acid gives upon reduction α-rhamnohexose which has been obtained by Fischer and Piloty* as small colorless crystals melting at 180° C. and showing levorotation, $[\alpha]_D = -61.4$ constant. The sugar exhibits mutarotation.

*Reactions.* — α-Rhamnohexose is reduced by sodium amalgam to its alcohol α-rhamnohexite ($[\alpha]_D = +14.0$). Oxidation with nitric acid splits off the methyl group and forms ordinary mucic acid; this reaction

* Ber., **23**, 3104, 3827.

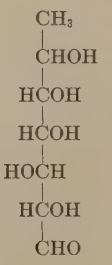
serves to establish the configuration of the sugar. α-Rhamnohexose-phenylosazone crystallizes in fine yellow needles insoluble in water, but soluble in hot alcohol; it melts at 200° C.

**β-Rhamnohexose.*** —



α-Rhamnohexonic acid upon heating with pyridine to 150° to 155°C. undergoes partial transformation to β-rhamnohexonic acid whose lactone is reduced by sodium amalgam to β-rhamnohexose. The sugar has not been isolated and its properties are for the most part unknown. Oxidation with nitric acid splits off the methyl group with formation of talomucic acid, this reaction serving to confirm the configuration. The phenylosazone of β-rhamnohexose melts at 200° C. and in all other respects is identical with that of α-rhamnohexose.

**α-Rhodeohexose.†** —



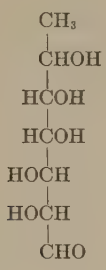
This sugar has been prepared from rhodeose in the same way that α-rhamnohexose was built up from rhamnose. The sugar consists of fine crystals melting at 125° to 126° C. and showing in aqueous solution $[\alpha]_D = +11.96$.

The phenylosazone consists of golden needles melting at 231° C.

* Fischer and Morrell, Ber., **27,** 382.

† Krauz, Ber., **43,** 482; Z. Zuckerind. Böhmen, **35,** 570.

**β-Rhodeohexose.*** —



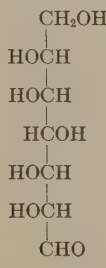
This sugar is formed from rhodeose in the same way as β-rhamnohexose from rhamnose. The sugar has not been isolated in the pure crystalline form.

## Heptoses

$C_7H_{14}O_7$

### aldoheptoses

**α-Glucoheptose.** —



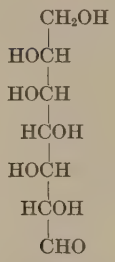
The sugar is formed by reducing the lactone of α-glucoheptonic acid. The sugar has been obtained by Fischer† in the form of large crystals melting at 180° to 190° C., and showing levorotation, $[\alpha]_D = -19.7$ (after solution $= -25$). The sugar is slightly sweet and is but slightly soluble in cold water (easily soluble in hot water). α-Glucoheptose reduces Fehling's solution but to a less extent than d-glucose. Reduction with sodium amalgam gives inactive α-glucoheptite and oxidation with nitric acid gives inactive α-glucopentoxypimelic acid.

α-Glucoheptose-phenylosazone forms fine yellow needles melting at 195° C.

* Krauz, Ber., **43,** 482; Z. Zuckerind. Böhmen, **35,** 570.

† Ann., **270,** 64.

**β-Glucoheptose.*** —

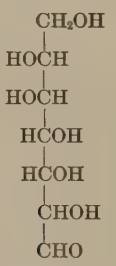


This sugar is formed by reducing the lactone of β-glucoheptonic acid, but has not been isolated as yet in the crystalline form.

Oxidation of β-glucoheptose with nitric acid gives the dibasic β-pentoxypimelic acid whose lactone, $C_7H_{10}O_8$, is dextrorotatory ($[\alpha]_D = +68.5$).

β-Glucoheptose-phenylhydrazone forms fine colorless needles melting at 190° to 193° C.; the phenylosazone of β-glucoheptose is in every respect identical with that of α-glucoheptose.

**d-Mannoheptose.** —



d-Mannoheptose was obtained by Fischer and Passmore† from d-mannose by addition of hydrocyanic acid, and reduction of the lactone of the resulting d-mannoheptonic acid. The sugar was obtained in the form of fine needles, melting at 134° to 135° C.; it has a sweet taste, is easily soluble in water but difficultly soluble in alcohol. The sugar is dextrorotatory showing mutarotation, $[\alpha]_D^{20} = +85$, 10 minutes after solution; $[\alpha]_D^{20}$ constant $= +68.64$. No perceptible fermentation was noted in presence of yeast.

*Identity of d-Mannoheptite and Perseite.* — Reduction of d-mannoheptose with sodium amalgam produces d-mannoheptite which was found by Fischer and Passmore to be identical with the natural heptite alcohol *perseite*, first found by Avequin‡ in the fruit of the alligator

* Fischer, Ann., **270**, 87. † Ber., **23**, 2226.

‡ Ann. chem. med. Ph. et Toxic., **7**, 467 (1831).

pear (*Persea gratissima*), and identified by Maquenne* in 1888. The relationship in properties of d-mannoheptite and perseite is shown in the following table (Fischer and Passmore):

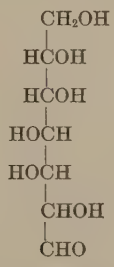
| | d-Mannoheptite (synthetic). | Perseite (natural). |
|---|---|---|
| Melting point | 188° C. | 188° C. |
| Melting point of heptacetyl compound | 119° C. | 119° C. |
| 100 parts of the saturated aqueous solution at 14° C. contain | 6.39 parts | 6.26 parts |
| Rotation of 0.4 gm. substance in 5 c.c. saturated borax solution in a 1 dcm. tube | +0.38° | +0.39° |

The relationship shown above was confirmed by the fact that perseite upon careful oxidation with nitric acid (1.14 sp. gr.) at 45° C. is changed to d-mannoheptose.

The identity established between perseite and mannoheptite is but one illustration of the increasing value which sugar synthesis has in the more refined problems of sugar analysis.

d-Mannoheptose-phenylhydrazone $C_7H_{14}O_6 : N_2HC_6H_5$ crystallizes in fine colorless needles melting at 197° to 200° C. The phenylosazone $C_7H_{12}O_5(N_2HC_6H_5)_2$ consists of fine yellow needles melting at 200° C.

**l-Mannoheptose.** —



l-Mannoheptose was obtained by Smith† from l-mannose, in the same manner as d-mannoheptose from d-mannose. The sugar was not obtained in the crystalline form. A 10 per cent solution of the sirup showed no fermentation with yeast. Reduction with sodium amalgam gave l-mannoheptite (m. p. 187° C.).

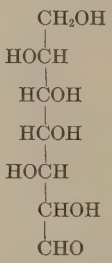
l-Mannoheptose-phenylhydrazone consists of colorless needles melting at 196° C.; the phenylosazone forms yellow needles (m. p. 203° C.).

**d, l-Mannoheptose** was obtained by Smith† by reducing the lactone of d, l-mannoheptonic acid. It was obtained only as an un-

* Compt. rend., **107**, 583. † Ann., **272**, 182.

fermentable optically inactive sirup. Reduction of the sugar gave d, l-mannoheptite melting at 203° C., which is higher than that observed for either of its components (187° C.). The same racemic compound was obtained by mixing equal parts of d-, and l-mannoheptite and allowing the aqueous solution to crystallize.

**α-Galaheptose.** —



α-Galaheptose was obtained by Fischer* from d-galactose by addition of hydrocyanic acid and reduction of the lactone of the resulting α-galaheptonic acid. The sugar was obtained only as a sweet, unfermentable, levorotatory sirup.

α-Galaheptose-phenylhydrazone was obtained as fine colorless needles melting at 200° C.; the phenylosazone consists of fine yellow needles. (m. p. 218° C.)

**β-Galaheptose** was obtained by Fischer* by reducing the lactone of β-galaheptonic acid, the latter being formed from d-galactose by addition of hydrocyanic acid at the same time as its isomer α-galaheptonic acid. The sugar has the same structure as α-galaheptose, excepting the H and OH groups in the second carbon atom which is opposite in the two sugars; the particular arrangement belonging to each sugar has not as yet been established.

β-Galaheptose crystallizes in the form of large prisms melting at 190° to 194° C. It has a sweet taste and is easily soluble in hot water. The sugar is levorotatory showing mutarotation, $[\alpha]_D = -22.5$ (10 minutes after solution) and $[\alpha]_D^{20}$ constant $= -54.4$.

**Volemose,** $C_7H_{14}O_7$. — This heptose sugar was obtained by Fischer† by oxidation of the naturally occurring heptite alcohol *volemite,* which was discovered by Bourquelot‡ in the fungus *Lactarius volemus.*

* Ann., **288,** 139.

† Ber., **28,** 1973.

‡ Bull. Soc. Mycol. de France, **5,** 132; Chem. Ztg., **15,** 190.

Volemose was obtained only in form of an impure sirup. The phenylosazone has the formula $C_7H_{12}O_5(N_2HC_6H_5)_2$ and consists of yellow crystals melting at 196° C.

The alcohol volemite $C_7H_{16}O_7$ consists of fine needles melting at 149° to 151° C., and showing in 10 per cent aqueous solution a dextrorotation of $[\alpha]_D + 1.92$.

The configurations of volemose and volemite have not as yet been established.

### KETOHEPTOSES

**Perseulose.** — $C_7H_{14}O_7$.

This ketoheptose was obtained by Bertrand* through the action of the sorbose bacterium upon perseite. The sugar was obtained in a pure crystalline form, the yield being about 45 per cent of the perseite taken.

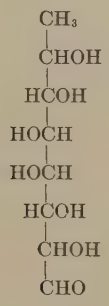
Perseulose is strongly levorotatory and exhibits mutarotation, $[\alpha]_D$ after solution $= -90$ and $[\alpha]_D^{25}$ constant $= -81°$.

Perseulose-phenylosazone, $C_7H_{12}O_5(N_2HC_6H_5)_2$, consists of yellow needles melting at 233° C.

### METHYLHEPTOSES

$CH_3 \cdot C_7H_{13}O_7$

**Rhamnoheptose.** —




This sugar was prepared by Fischer and Piloty† by addition of hydrocyanic acid to $\alpha$-rhamnohexose and reduction of the lactone of the resulting rhamnoheptonic acid. Rhamnoheptose was obtained only as a colorless sweet sirup of weak dextrorotation, ($[\alpha]_D = +8.4$ about).

The phenylhydrazone of rhamnoheptose is characterized by low

* Compt. rend., **147,** 201; **149,** 225.

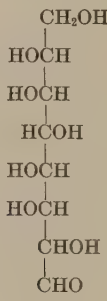
† Ber., **23,** 3102.

solubility in water and separates with great readiness. It consists of colorless needles having the composition $C_8H_{16}O_6N_2HC_6H_5$ and melting at 200° C. The phenylosazone, $C_8H_{14}O_5(N_2HC_6H_5)_2$, consists of fine yellow needles difficultly soluble in water and hot alcohol; its melting point is about 200° C.

## Octoses

$C_8H_{16}O_8$

**α-Glucooctose.** —



This sugar was synthetized by Fischer* from α-glucoheptose; the latter by addition of hydrocyanic acid yields 2 stereo-isomers, α- and β-glucooctonic acid. The lactone of α-glucooctonic acid gives upon reduction α-glucooctose.

α-Glucooctose crystallizes from water in fine white needles as a hydrate having the formula $C_8H_{16}O_8 + 2\,H_2O$. The sugar is levorotatory and shows mutarotation; $[\alpha]_D^{20} = -\,50.5$ (constant for the anhydride $C_8H_{16}O_8$).

α-Glucooctose has a sweet taste and gives all the ordinary reaction of a reducing sugar.

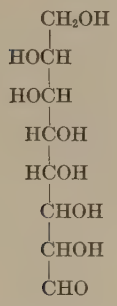
α-Glucooctose-phenylhydrazone, $C_8H_{16}O_7N_2HC_6H_5$, separates very readily as a difficultly soluble compound, consisting when pure of fine colorless needles melting at about 190° C. The phenylosazone forms fine yellow crystals melting at 210° to 212° C. and having the composition $C_8H_{14}O_6(N_2HC_6H_5)_2$.

Upon reduction with sodium amalgam α-glucooctose gives its alcohol α-glucooctite, $C_8H_{18}O_8$, which melts at 141° C.

**β-Glucooctose**, formed by reducing the lactone of β-glucooctonic acid, has not been studied.

* Ann., **270**, 64.

**d-Mannooctose.** —

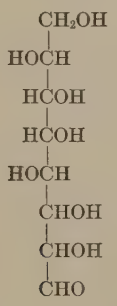


This sugar was built up from d-mannoheptose by Fischer and Passmore* by addition of hydrocyanic acid and reducing the lactone of the resulting d-mannooctonic acid. The sugar was obtained only as a sweet colorless unfermentable sirup with slight levorotation, ($[\alpha]_D = -3.3$).

d-Mannooctose upon reduction gives d-mannooctite $C_8H_{18}O_8$, which consists of colorless very difficultly soluble crystals melting at 258° C.

The phenylhydrazone $C_8H_{16}O_7N_2HC_6H_5$ forms colorless needles very insoluble in water and melting, when quickly heated, at about 212° C. The phenylosazone forms fine yellow needles very insoluble in hot water and alcohol, and melting at about 223° C.

**α-Galaoctose.** —



α-Galaoctose was built up by Fischer† from α-galaheptose by adding hydrocyanic acid and reducing the lactone of the resulting α-galaoctonic acid. The sugar was obtained as colorless crystals of the monohydrate $C_8H_{16}O_8 + H_2O$ melting at 109° to 111° C. The sugar is levorotatory, $[\alpha]_D = -40°$ about.

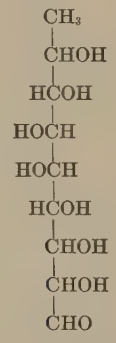
* Ber., **23,** 2226.
† Ber., **27,** 3198.

$\alpha$-Galaoctose gives upon reduction $\alpha$-galaoctite $C_8H_{18}O_8$ which consists of colorless needles melting at 220° to 225° C. The phenylhydrazone of $\alpha$-galaoctose has the formula $C_8H_{16}O_7N_2HC_6H_5$ and melts at 200° to 205° C. The osazone $C_8H_{14}O_6(N_2HC_6H_5)_2$ forms fine yellow needles melting at 220° to 225° C.

## Methyloctoses

$CH_3 \cdot C_8H_{15}O_8$

**Rhamnooctose. —**

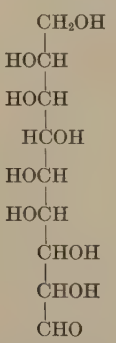


This sugar was prepared by Fischer and Piloty* from rhamnoheptose by adding hydrocyanic acid and reducing the lactone of the resulting rhamnooctonic acid. The sugar was not separated in the pure condition and its properties have not been determined.

## Nonoses

$C_9H_{18}O_9$

**$\alpha$-Gluconononose. —**



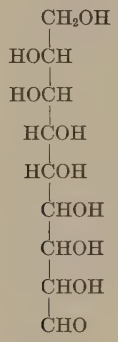
* Ber., **23**, 3102.

α-Glucononose was prepared by Fischer* from α-glucooctose by adding hydrocyanic acid and reducing the lactone of the resulting α-gluconononic acid. The sugar was obtained only as a colorless non-fermentable sirup with slight dextrorotation.

Reduction of α-glucononose gives the alcohol α-glucononite, $C_9H_{20}O_9$, which consists of colorless crystals melting at 194° C.

α-Glucononose-phenylhydrazone $C_9H_{18}O_8N_2HC_6H_5$ forms white needles only slightly soluble in cold water and alcohol and melting at about 194° C. The phenylosazone $C_9H_{16}O_7(N_2HC_6H_5)_2$ consists of fine yellow needles almost insoluble in hot water and alcohol, and melting at 220° to 223° C.

**d-Mannononose.** —



d-Mannononose was prepared by Fischer and Passmore† from d-mannooctose by adding hydrocyanic acid and reducing the lactone of the resulting d-mannonononic acid. The sugar was obtained as white crystals melting at 130° C. and showing in aqueous solution dextrorotation ($[\alpha]_D^{20} = +$ 50 about).

d-Mannononose is fermented by yeast with the same ease and completeness as d-glucose.

d-Mannononose-phenylhydrazone, $C_9H_{18}O_8N_2HC_6H_5$, forms crystals easily soluble in hot water and melting at 223° C. The phenylosazone $C_9H_{18}O_7(N_2HC_6H_5)_2$, forms yellow needles almost insoluble in hot water and alcohol and melting at 217° C.

A peculiarity of d-mannononose is its striking resemblance to d-glucose. The resemblance in composition, melting point, specific rotation and fermentability could easily cause confusion; an analysis of the osazone easily serves, however, to fix the class of the sugar (see page 371).

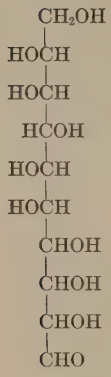
* Ann., **270**, 64.

† Ber., **23**, 2226.

## Decoses

$C_{10}H_{20}O_{10}$.

**α-Glucodecose.** —



This sugar has recently been prepared by Phillippe* from α-gluconose, following the usual method of adding hydrocyanic acid, saponifying and reducing the lactone of the resulting α-glucodeconic acid by means of sodium amalgam.

α-Glucodecose crystallizes in needle-shaped crystals, which show in aqueous solution a dextrorotation, $[\alpha]_D^{20} = +50.4$ (constant); in fresh solution $[\alpha]_D^{20} = +37$. Under certain conditions the sugar may crystallize in plates having one molecule of water of crystallization. The sugar reduces Fehling's solution about 76 per cent as strongly as glucose; it forms a phenylhydrazone melting at about 278° C.

α-Glucodecose is reduced by sodium amalgam to the corresponding alcohol α-glucodecite, which consists of prismatic needles, melting and subliming at 222° C. and showing in aqueous solution $[\alpha]_D^{18} = +1.2$.

* Compt. rend., **151,** 986; **152,** 1774.

# CHAPTER XX

## THE DISACCHARIDES

### Dipentose Saccharides

$$O\begin{cases}C_5H_9O_4\\C_5H_9O_4\end{cases}$$

**Diarabinose,** $C_{10}H_{18}O_9$. — This disaccharide was obtained by O'Sullivan* in heating Gedda gum with sulphuric acid (0.3 – 0.5 per cent). Its formation is probably due to the breaking down of higher condensation substances of the gum (arabinic acids) into diarabinose and other hydrolytic products. Diarabinose (also called arabinon, arabiose and arabinobiose) was obtained by O'Sullivan as an amorphous vitreous hygroscopic substance of sweet taste and very soluble in water from which it is precipitated by strong alcohol. The product melts at about 75° to 80° C., and is strongly dextrorotatory, $[\alpha]_D = +\ 198.8$. It reduces Fehling's solution about 58.8 per cent as strong as d-glucose. Analysis of the sugar and determination of its molecular weight by the freezing point method indicate the formula $C_{10}H_{18}O_9$.

Upon heating with 2 per cent sulphuric acid, diarabinose is hydrolyzed quantitatively into l-arabinose.

$$\underset{\text{Diarabinose}}{C_{10}H_{18}O_9} + H_2O = 2\,\underset{\text{l-Arabinose.}}{C_5H_{10}O_5}.$$

### Pentose–hexose Saccharides.

$$O\begin{cases}C_5H_9O_4\\C_6H_{11}O_5\end{cases}$$

**Glucoapiose,** $C_{11}H_{20}O_{10}$. — This disaccharide has not been isolated as yet although its presence has been recognized by Vongerichten† among the constituents of the glucoside apiin, which is obtained from parsley. The formation of glucoapiose from apiin should proceed according to the following equation:

$$\underset{\text{Apiin}}{C_{26}H_{28}O_{14}} + H_2O = \underset{\text{Glucoapiose}}{C_{11}H_{20}O_{10}} + \underset{\text{Apigenin.}}{C_{15}H_{10}O_5}.$$

* J. Chem. Soc., **57,** 59; **59,** 1029.

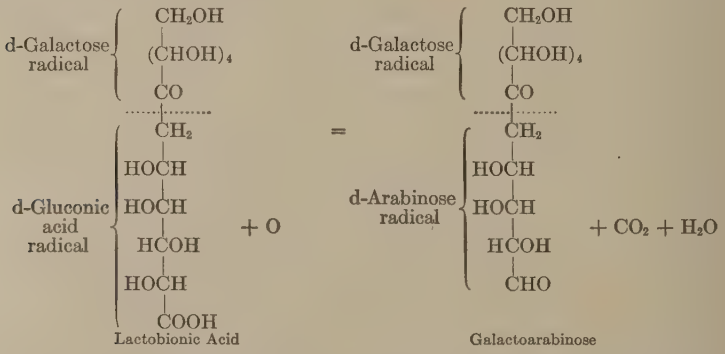
† Ber., **9,** 1124; **33,** 2334, 2904.

The glucoapiose, however, is itself decomposed by the hydrolytic agent into glucose and apiose (p. 544).

$$\underset{\text{Glucoapiose}}{C_{11}H_{20}O_{10}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Apiose.}}{C_5H_{10}O_5},$$

so that the separation of the disaccharide itself has not been accomplished by this means.

**Galactoarabinose,** $C_{11}H_{20}O_{10}$. — This sugar has not been found as yet in nature. It has been prepared synthetically by Ruff and Ollendorf* from ordinary lactose, by first oxidizing the sugar by means of bromine to lactobionic acid and then oxidizing the calcium salt of the latter with hydrogen peroxide in presence of basic ferric acetate; the COOH group of the acid is thus destroyed and a disaccharide sugar obtained with 11 C atoms.




The process is similar to those previously described by which the monobasic acids of sugars are degraded into sugars of one less carbon atom. (See under d-erythrose, page 540).

Galactoarabinose has been obtained only as a colorless dextrorotatory sirup. Upon heating with dilute acids it is hydrolyzed into d-galactose and d-arabinose.

$$\underset{\text{Galactoarabinose}}{C_{11}H_{20}O_{10}} + H_2O = \underset{\text{d-Galactose}}{C_6H_{12}O_6} + \underset{\text{d-Arabinose.}}{C_5H_{10}O_5}.$$

### Methylpentose-hexose Saccharides

$$O\begin{matrix}/\ C_5H_8O_4\cdot CH_3 \\ \backslash\ C_6H_{11}O_5\end{matrix}$$

No sugar of the constitution $C_{12}H_{22}O_{10}$ has as yet been discovered. A methyl glucoside of *mannorhamnose,* however, has been isolated.

* Ber., **32,** 552; **33,** 1806.

**Methyl mannorhamnoside,*** $C_{12}H_{21}O_{10} \cdot CH_3$. — This glucoside has been obtained by hydrolysis of strophanthin, the poisonous principle of the seeds of *Strophanthus Kombé*, used by the natives of eastern Africa as an arrow poison. Strophanthin is decomposed by dilute acids as follows:

$$\underset{\text{Strophanthin}}{C_{40}H_{66}O_{19}} = \underset{\text{Strophanthidin}}{(C_{27}H_{38}O_7 + 2\,H_2O)} + \underset{\text{Methyl mannorhamnoside.}}{C_{12}H_{21}O_{10} \cdot CH_3}.$$

One part of strophanthin is dissolved in 5 parts of cold 0.5 per cent hydrochloric acid and then warmed for some time at 70° to 75° C. and then at 75° to 80° C. The strophanthidin which crystallizes out is filtered off and the cold filtrate freed from hydrochloric acid by means of silver oxide. The clear filtered solution is then concentrated in a vacuum to a sirup from which the methyl mannorhamnoside can be precipitated by means of ether. The compound upon recrystallizing from alcohol is obtained as white crystals melting at 207° C. The glucoside is easily soluble in water, fairly soluble in hot alcohol, but almost insoluble in ether. It is dextrorotatory ($[\alpha]_D = +8.24$ about), unfermentable and does not reduce Fehling's solution. Upon heating with an excess of strong mineral acid, methyl mannorhamnoside yields large amounts of methylfurfural and levulinic acid. The glucoside is hydrolyzed by heating with 5 parts of 1 per cent sulphuric acid as follows:

$$\underset{\text{Methyl mannorhamnoside}}{C_{12}H_{21}O_{10} \cdot CH_3} + 2\,H_2O = \underset{\text{Mannose}}{C_6H_{12}O_6} + \underset{\text{Rhamnose}}{C_6H_{12}O_5} + \underset{\text{Methyl alcohol.}}{CH_3OH}.$$

## Dihexose Saccharides

$$O\begin{cases} C_6H_{11}O_5 \\ C_6H_{11}O_5 \end{cases}$$

This group, by far the most important of the higher saccharides, includes the three well-known sugars: sucrose, maltose and lactose.

**Sucrose.** — Saccharose. Cane sugar.

$C_{12}H_{22}O_{11}$.

**Occurrence.** — Sucrose occurs very widely distributed throughout the vegetable kingdom; from its importance as a commodity and food product it is the best known of the sugars.

The approximate distribution of sucrose in different fresh plant materials is as follows:

| | Per cent. |
|---|---|
| Juice of green leaves | 0.1 — 2.0 |
| Juice of stalks from maize, sugar cane, etc. | 2.0 — 20.0 |
| Sap of maple, birch, palm and other trees | 1.0 — 5.0 |
| Apples, berries, oranges, prunes, bananas and other fruits | 0.5 — 14.0 |
| Seeds, grains, nuts, etc. | 0.5 — 12.0 |
| Buds, blossoms and flowering organs | 0.1 — 15.0 |
| Roots, yams, bulbs, tubers, rhizomes, etc. | 0.5 — 25.0 |

* Feist, Ber., **31**, 535; **33**, 2063, 2069, 2091.

Sucrose has not been identified with certainty in any products of purely animal origin. It occurs in honey in amounts ranging usually from 0.0 to 10 per cent; in abnormal cases the percentage of sucrose may exceed 10 per cent. The sucrose of honey, however, is derived primarily from floral nectar or other plant sources and must therefore be regarded as of vegetable origin.

**Preparation of Sucrose.** *Technical Processes.* — The sugar cane, sugar beet, maple, palm, sorghum and maize have all been utilized for the production of sugar. The annual production of raw sucrose for the world at present is about 16,000,000 long tons (1 long ton = 2240 lbs.) of which about 8,500,000 tons are made from sugar cane and about 7,500,000 tons from the sugar beet; the production from other sources is insignificant. In the manufacture of raw sugar the juice is extracted from the sugar cane by means of mills, and from the sugar beet by means of diffusion batteries. The extracted juice is then clarified* usually with milk of lime, any excess of the latter being removed by means of carbon dioxide ("carbonatation"), sulphurous acid ("sulphitation"), phosphoric acid or other precipitating agent. The clarified juice, which may contain from 10 to 18 per cent of sucrose, is then evaporated to crystallization. In primitive countries the evaporation is done in open pans directly over the fire; in the more modern factories some form of vacuum evaporator is used. After the evaporated juice has crystallized, the thick magma of crystals ("massecuite" or "fillmass") is purged from its mother liquor, or molasses, a process which is usually carried out in centrifugals; the product thus obtained constitutes the raw sugar of commerce and varies in purity from 80 per cent to almost 100 per cent pure sucrose.

*Refining.* — The raw sugar of commerce is afterwards refined. The process of refining comprises usually (1) washing the crystals of raw sugar with concentrated sirups to remove adhering molasses, a process sometimes termed "affining," (2) dissolving the purified crystals in hot water and clarifying the solution with lime or other agents; (3) filtering the clarified solution over bone black† to remove coloring matter and other impurities; (4) evaporating the filtered and decolorized solution to a magma of crystals; (5) centrifuging the "massecuite" or "fillmass" and drying the pure white crystals of sucrose in

* The number of substances which have been proposed for clarifying sugar juices is almost unlimited. A classification of clarifying agents made by Lippmann (Die Deutsche Zuckerind., **34,** 9) includes 620 different materials or processes.

† The use of bone black has been largely discontinued in the refining of beet sugar.

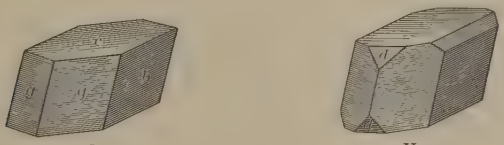
granulators, or in cones, cubes, dominos or other forms according to the demands of the trade. Refined sugar is usually about 99.8 to 99.9 per cent pure, the remaining 0.1 to 0.2 per cent consisting mostly of moisture with occasional traces of ash, invert sugar, raffinose and caramel substances.

To obtain sucrose perfectly pure the best grade of refined sugar is recrystallized from neutral redistilled 96 per cent alcohol. The method described upon page 121 may be used to advantage.

*Isolation of Sucrose from Plant Substances.* — For the separation of sucrose from plant substances, when only small amounts of the sugar are present, Schulze* has made use of the difficultly soluble strontium bisaccharate $C_{12}H_{22}O_{11} \cdot 2\,SrO$. The fresh material, in presence of an excess of pure finely powdered calcium carbonate to neutralize any acidity, is extracted with hot 90 per cent alcohol. After cooling, the extract is filtered and then heated to boiling with the addition of a hot saturated solution of strontium hydrate using over 3 parts of $Sr(OH)_2$ for every 1 part of sucrose supposed to be present. After boiling 30 minutes the precipitate is filtered, washed with alcohol and again boiled for 30 minutes with strontium hydrate solution. The precipitate is filtered hot, using a hot water funnel, and then, after suspending in water, decomposed with a stream of carbon dioxide. The solution is filtered from strontium carbonate and then evaporated to a sirup which is purified by means of neutral 95 per cent alcohol in the usual way. The alcoholic solution is reëvaporated to a sirup and repurified as before, the process of evaporation and extraction of the sirup with alcohol being repeated several times. The final sirup is placed over concentrated sulphuric acid in a cool place for crystallization.

### PROPERTIES OF SUCROSE

**Crystalline Form.**—Sucrose crystallizes in beautiful colorless crys-



I II

Fig. 195. — Monoclinic crystals of sucrose. I, Tabular form; II, Form with hemihedral faces.

tals belonging to the monoclinic system. The crystals have hemihedral surfaces and show the greatest variety of form (Fig. 195). The shape

* Z. Ver. Deut. Zuckerind., **38**, 221.

of sucrose crystals is greatly modified by other substances, the effect of raffinose in this respect being especially pronounced (p. 735). Crystals of sucrose may take up soluble coloring matter from the mother liquor during growth and such crystals often show a variation in tint when viewed in different directions (*pleochroism*). Although sucrose in solution is optically active, its crystals, as was first noted by Biot,* do not rotate the plane of polarized light.

**Melting Point and Specific Gravity.** — The melting point of sucrose is given by different observers as varying between 160° to 180° C., the variations being due apparently to differences in method and in the physical character of the sugar. The specific gravity of solid sucrose is given by different authorities as between 1.58 and 1.61, the differences being probably due to variations in the character of the crystals. The recent determinations of Plato† give for chemically pure sucrose $d\,\frac{15^\circ}{15^\circ} = 1.591$. The specific gravity of the hypothetical solid sucrose in aqueous solution is given by Plato as $d\,\frac{15^\circ}{15^\circ} = 1.55626$; the difference between this figure and that for the actual solid being due to the contraction in volume during solution. The part which this phenomenon plays in the determination of sucrose by densimetric methods has already been considered (p. 33).

**Solubility.** — The solubility of sucrose in water of different temperatures and the character of the solutions thus obtained are given by Herzfeld‡ in Table XCI.

Sucrose is soluble in 80 parts of boiling absolute alcohol, more easily soluble in dilute alcohol but insoluble in ether.

### SOLUBILITY OF SUCROSE AND THE MELASSIGENIC ACTION OF SALTS

The solubility of sucrose, as of all other sugars, is affected to a marked degree by the presence of foreign organic and inorganic substances. Such impurities play an important part technically in preventing the recovery of sucrose from sugar-house molasses. A saturated solution of sucrose in contact with sucrose crystals can dissolve no more sucrose at constant temperature; if solid potassium acetate, or sodium chloride, or many other salts be stirred into the solution, however, it will not only be dissolved but more of the sucrose will also enter solution. In other words more sugar will be dissolved than can be held in solution by the water alone. This phenomenon is explained by many authorities as being due to the formation of sucrose-salt com-

* Mémoires de l'Académie, **13,** 59, 126. † Z. Ver. Deut. Zuckerind., **50,** 1012.
‡ Z. Ver. Deut. Zuckerind., **42,** 181, 232.

pounds, or complexes, which have a greater solubility than the sucrose alone.

TABLE XCI.

*Solubility of Sucrose in Water at Different Temperatures.*

| Temperature. | Grams sucrose in 100 grams solution. | Grams sucrose dissolved by 100 grams water. | Grams water corresponding to 1 gram dissolved sucrose. | Specific gravity of solution, 17.5° C. |
|---|---|---|---|---|
| Deg. C. | | | | |
| 0 | 64.18 | 179.2 | 0.5580 | 1.31490 |
| 5 | 64.87 | 184.7 | 0.5414 | 1.31920 |
| 10 | 65.58 | 190.5 | 0.5249 | 1.32353 |
| 15 | 66.30 | 197.0 | 0.5076 | 1.32804 |
| 20 | 67.09 | 203.9 | 0.4904 | 1.33272 |
| 25 | 67.89 | 211.4 | 0.4730 | 1.33768 |
| 30 | 68.70 | 219.5 | 0.4556 | 1.34273 |
| 35 | 69.55 | 228.4 | 0.4378 | 1.34805 |
| 40 | 70.42 | 238.1 | 0.4200 | 1.35353 |
| 45 | 71.32 | 248.7 | 0.4021 | 1.35923 |
| 50 | 72.25 | 260.4 | 0.3840 | 1.36515 |
| 55 | 73.20 | 273.1 | 0.3662 | 1.37124 |
| 60 | 74.18 | 287.3 | 0.3418 | 1.37755 |
| 65 | 75.18 | 302.9 | 0.3301 | 1.38404 |
| 70 | 76.22 | 320.5 | 0.3120 | 1.39083 |
| 75 | 77.27 | 339.9 | 0.2942 | 1.39772 |
| 80 | 78.36 | 362.1 | 0.2762 | 1.40493 |
| 85 | 79.46 | 386.8 | 0.2585 | 1.41225 |
| 90 | 80.61 | 415.7 | 0.2406 | 1.41996 |
| 95 | 81.77 | 448.6 | 0.2229 | 1.42778 |
| 100 | 82.97 | 487.2 | 0.2053 | 1.43594 |

**Solubility of Sucrose in Beet Molasses.** — A condition similar to that previously described exists in sugar-beet molasses as is shown by the following analysis:

| | Per cent. |
|---|---|
| Water | 20 |
| Sucrose | 50 |
| Salts | 10 |
| Reducing sugars | trace |
| Organic non-sugars | 20 |

The 20 parts of the water alone could hold in solution at ordinary temperature only about 40 parts of sucrose, so that if the salts and other impurities were absent sucrose would begin to crystallize. Such a removal of salts is the principle of the old osmose process for recovering sucrose from beet molasses first devised by Dubrunfaut. If beet molasses be dialyzed by means of parchment paper against running water the salts will diffuse with much greater rapidity than the sucrose and in this way the percentage of melassigenic impurities can be considerably reduced; beet molasses thus purified will deposit upon evaporation crystals of sucrose up to the new saturation point for the

solution of undialyzed impurities. This process has given place technically to the saccharate process of sucrose recovery to be described later.

**Solubility of Sucrose in Cane Molasses.** — In low-grade sugar-cane molasses an opposite condition exists to that in beet molasses; in cane molasses the amount of sucrose is less than that which will saturate the quantity of water present. This is shown by the following analysis of a low-grade cane molasses.

| | Per cent. |
|---|---|
| Water | 20 |
| Sucrose | 30 |
| Invert sugar | 30 |
| Salts | 8 |
| Organic non-sugars | 12 |

**Geerligs's Theory of Melassigenic Action.** — A molasses of the above composition can dissolve no more sucrose, yet the 20 parts of water alone could hold in solution 40 parts of sucrose at ordinary temperature. This difference in behavior upon the part of cane molasses is explained by Prinsen Geerligs * as due to a combination between the invert sugar and the salts of the molasses (the potassium organic salts more especially). The invert-sugar-salt complexes which are thus formed hold in combination a large amount of water of hydration which thus reduces the quantity of water available for solution of the sucrose. The power of sucrose to form salt complexes is much less than that of invert sugar so that it is only in cane molasses of very low invert sugar content that sucrose-salt complexes exist in sufficient quantity to raise the solubility of sucrose above the saturation point of the water present.

According to this theory the addition of anhydrous glucose to a saturated solution of a sucrose-salt complex should displace the sucrose and cause a part of the latter to crystallize out. This was verified experimentally by Geerligs who found that when 225 gms. of anhydrous glucose were added to 300 gms. of a saturated solution of sucrose and potassium acetate, and the mixture allowed to stand for several months 75 gms. of sucrose separated in the crystalline form. A check solution without addition of glucose showed no evidence of crystallization.

The melassigenic action of different organic and mineral substances upon sucrose has been studied by many investigators and for a complete review of the various physical and chemical theories upon the subject the student is referred to the works of Lippmann † and Geerligs.‡

* Z. Ver. Deut. Zuckerind, **45**, 320.

† " Chemie der Zuckerarten," 1147–1162.

‡ " Cane Sugar and its Manufacture " (1909), 301–317.

**Boiling Point of Sucrose Solutions.** — The boiling point of aqueous sucrose solutions of different concentrations is given by Gerlach * as follows:

| Per cent sucrose .... | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90.8 |
|---|---|---|---|---|---|---|---|---|---|
| Boiling point °C .... | 100.4 | 100.6 | 101.0 | 101.5 | 102.0 | 103.0 | 106.5 | 112.0 | 130.0 |

**Specific Rotation.** — The specific rotation of sucrose has been the subject of greater study than that of any other sugar. The first determinations were made in 1819 by Biot,† who first introduced the constant of specific rotation and thereby founded the science of optical analysis.

The value for the specific rotation of sucrose in aqueous solution is very closely $[\alpha]_D^{20} = +66.5$. The influences of temperature, concentration, solvents, salts, etc., upon the specific rotation of sucrose have already been considered.

**Fermentations of Sucrose.** *Alcoholic Fermentation.* — In so far as the various yeasts, moulds and bacteria secrete the enzyme *invertase* they are able to ferment sucrose in the same manner as its products of inversion, glucose and fructose. The majority of the yeasts secrete invertase and ferment sucrose with the same yield of alcohol and carbon dioxide as is obtained from glucose and fructose; the process is somewhat slower, however, in its first stages owing to the retarding effect of the inversion which must precede fermentation.

*Non-inverting Yeasts and Moulds.* — A considerable number of alcohol-producing organisms, such as *Saccharomyces octosporus*,‡ *Saccharomyces apiculatus*,§ and most of the Mucor genus of moulds do not secrete invertase and pure cultures of these do not ferment sucrose. Attempts have been made to employ organisms of this class such, for example, as *Mucor circinelloides*,‖ for destroying the invert sugar of cane molasses, in the hope of obtaining the residual sucrose in a suitable condition for recovery. The process has not been a technical success.¶

* Z. Ver. Deut. Zuckerind, **13,** 283.

† Mémoires de l'Académie, **2,** 41; **13,** 118.

‡ Fischer and Lindner, Ber., **28,** 984.

§ Fischer and Lindner, Ber., **28,** 3034.

‖ Gayon, Ann. chim. phys. [3], **14,** 258.

¶ Upon the basis of Prinsen Geerligs's molasses theory it is evident that fermenting away the invert sugar of cane molasses would have but little effect upon rendering the sucrose more crystallizable. The result would simply be to change the molasses from a cane to a beet type. Suppose a cane molasses of the following composition to have its invert sugar fermented and the solution of sucrose, salts

*Lactic and Butyric Fermentations.* — The lactic and butyric acid fermentations can be produced with sucrose by the same organisms which produce these fermentations with d-glucose and d-fructose. In a few cases, however, where the particular organism does not secrete invertase, fermentation of sucrose does not take place.

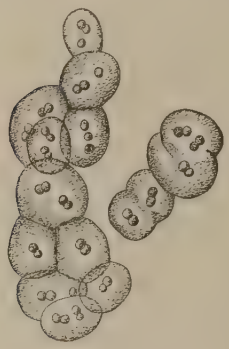


Fig. 196. — Leuconostoc mesenterioides. After Zopf. (48-hour culture in molasses showing slimy envelopes of dextran.)

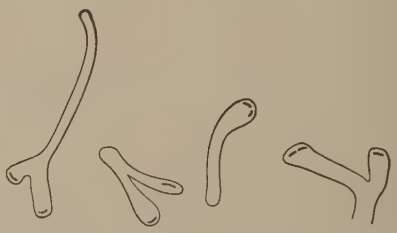


Fig. 197. — Bacterium pediculatum. After Koch and Hosaeus.

*Viscous Fermentation.* — One of the most common fermentations of sucrose observed in sugar factory experience is the so-called viscous fermentation by which sucrose is converted into the gum dextran. The best known dextran-producing organism is the *Leuconostoc mesen-*

and non-sugars to be evaporated to the original concentration. We would then have:

| | *A* Original cane molasses. | *B* Molasses after fermentation of invert sugar. | *C* *B* with 8 parts of water evaporated. | *D* Percentage composition of residue C. |
|---|---|---|---|---|
| | Per cent. | Parts. | Parts. | Per cent. |
| Water | 20 | 20 | 12 | 20 |
| Sucrose | 30 | 30 | 30 | 50 |
| Invert sugar | 32 | 0 | 0 | |
| Salts | 6 | 6 | 6 | 10 |
| Organic non-sugars | 12 | 12 | 12 | 20 |
| Total | 100 | 68 | 60 | 100 |

The composition of the evaporated residue after fermenting away the invert sugar is proximately the same as beet molasses from which, as has been seen (p. 649), no sucrose will crystallize.

*terioides* (Fig. 196), which was supposed by the earliest investigators to ferment sucrose according to the following equation:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} = \underset{\text{Dextran}}{C_6H_{10}O_5} + \underset{\text{Fructose.}}{C_6H_{12}O_6}.$$

Later researches * showed, however, that the action of Leuconostoc and of many other "dextran-formers" consisted first in an inversion of the sucrose into d-glucose and d-fructose so that the above formula is not strictly correct; it was also established that dextran is a polysaccharide $(C_6H_{10}O_5)_n$ and that it constitutes the slimy jelly-like capsule in which the organisms are embedded. The dextran is, therefore, to be regarded of assimilative, rather than of fermentative (i.e., enzymic) origin. Very similar to Leuconostoc in its action is the *Bacterium pediculatum* discovered by A. Koch and Hosaeus in the sirup of a sugar factory. The organism secretes a slimy capsule of gum which, becoming greatly elongated upon one side, gives it a stem-like appearance (Fig. 197).

Certain gum-producing organisms have been found, such as *Micrococus gelatigenosus*, *Bacillus gummosus*, *Bacterium gummosum*, etc., which form dextran from sucrose but not from glucose. This has been regarded as a fermentation of sucrose without preceding inversion; most of the members of this class of organisms are found, however, to secrete invertase so that the sucrose in these, and no doubt in all other cases, where fermentation or assimilation takes place, is probably first inverted. The peculiarity which certain bacteria have of forming dextran from sucrose but not from glucose may be explained by supposing that these organisms are able to ferment or assimilate glucose only at the time of its separation from the sucrose molecule (i.e., in its nascent state) and not after it is already formed. Even in the case of Leuconostoc, which can assimilate free glucose and fructose, the formation of dextran is several times more rapid with sucrose.†

*Formation of Mannite During Fermentation.* — In the so-called viscous or gummy fermentation of sucrose mannite is frequently formed in addition to dextran. Monoyer ‡ regarded the two substances as products of separate fermentations which he formulated as follows:

Mannitic fermentation:

$$\underset{\text{Sucrose}}{13\ C_{12}H_{22}O_{11}} + 25\ H_2O = \underset{\text{Mannite.}}{24\ C_6H_{14}O_6} + 12\ CO_2. \qquad (1)$$

* For a full account of the action of Leuconostoc upon sucrose see the work of Liesenberg and Zopf, Centralbl. für Bakteriologie, **12,** 659; **13,** 339.

† Prinsen Geerligs "Cane Sugar and its Manufacture" (1909), 38.

‡ Thèse pour le doctorat en médecine, Strasbourg, 1862.

Gum fermentation:

$$\underset{\text{Sucrose.}}{12\ C_{12}H_{22}O_{11}} = \underset{\text{Gum.}}{12\ C_{12}H_{20}O_{10}} + 12\ H_2O. \qquad (2)$$

According to the above combined equations 100 parts of sucrose yield 45.5 parts of gum and 51.1 parts of mannite. This proportion is not fixed, however, the variation in yield being explained by the predominence of one or the other fermentation. It is more probable, however, that the dextran is formed as an assimilative and the mannite as a reduction product in many anaërobic fermentations by a single species of bacteria.

The gum, which is produced in the viscous fermentation of sucrose, is not always dextran. It may also consist of levulan or levan (p. 615), which give fructose upon hydrolysis, whereas dextran is hydrolyzed only into glucose.

*Influence of Fermentation Gums Upon Polarimetric Determination of Sucrose.* — The presence of highly dextrorotatory and levorotatory gums in sugar-house products may introduce a considerable error in the polarimetric estimation of sucrose. Browne* reported the following analyses of badly fermented sugar-cane juices:

| Degrees Brix. | Polarization. | Sucrose. | Reducing sugars. | Dextran. | Apparent purity coefficient. |
|---|---|---|---|---|---|
| | | Per cent. | Per cent. | Per cent. | |
| 7.8 | +18.0 | 0.0 | 0.15 | 5.90 | 232 |
| 4.8 | +10.4 | 0.0 | trace | 3.35 | 216 |

The presence of dextran in cane sirups and molasses might cause the chemist to suspect an adulteration with commercial glucose or starch sirup. In such cases the gum should be precipitated with strong alcohol, then, after decanting the clear solution, dissolved in a small amount of water and a drop or two of iodine solution added; a red coloration, indicative of erythrodextrin, will appear if starch sirup has been used as an adulterant. Dextran does not respond to this test.

According to Taggart† the presence of the gum levan in sugar products may also introduce a considerable error in the polarimetric estimation of sucrose.

*Cellulosic Fermentation.* — Some varieties of bacteria assimilate sucrose with formation of cellulose. The *Bacterium xylinum* (sorbose bacterium) thrives in sucrose solutions and this organism according to A. J. Brown‡ forms cellulose. Browne§ found in the cane juices of Louisiana an organism which formed white gelatinous lumps of cellu-

* J. Am. Chem. Soc., **28**, 462.
† J. Ind. Eng. Chem., **3**, 646.
‡ J. Chem. Soc., **49**, 432.
§ J. Am. Chem. Soc., **28**, 463.

lose, weighing in some cases several pounds. The product after purifying with hot sodium hydroxide was colored blue with zinc chloride and iodine, was soluble in ammoniacal copper solution and had the composition of cellulose. The amount of cellulose formed by the organism was about 7 per cent of the total sucrose destroyed.

*Citric Fermentation.* — The citric fermentation (p. 585) may also occur with sucrose, the fungus *Citromyces glaber* yielding 50 per cent of the sucrose in citric acid. A Citromyces found by Browne * upon hot-room molasses in Louisiana was found to contain over 11 per cent chitin; the latter gave upon hydrolysis with hydrochloric acid over 60 per cent of pure glucosamine chloride (p. 753).

Among other fermentation products of sucrose, besides those already mentioned, may be mentioned butyl and amyl alcohols and acetaldehyde; formic, acetic, butyric, propionic, valeric, capronic, caprylic, lactic and succinic acids; as well as the gaseous products hydrogen and methane. For a description of the fermentations which give rise to these and other substances the student is referred to the works of Lafar,† Jörgensen ‡ and Lippmann.§

### Decomposition of Sucrose by Heating

Sucrose upon heating above its melting point begins to decompose with evolution of water. Between 170° and 190° C a mixture of brownish colored substances, known as caramel, is formed; above 190° C. large quantities of carbon dioxide and monoxide are given off together with various volatile decomposition products such as aldehyde, acetone, acrolein, furfural and even benzolderivatives, as benzaldehyde. From a technical viewpoint the most important of these decomposition products is caramel.

**Caramel** is usually prepared by heating sucrose to 170° to 190° C. and consists of a mixture of decomposition products, the exact composition of which has not been fully ascertained. The caramelization or browning of sucrose may begin, however, at temperatures below 100° in presence of moisture. As ordinarily prepared from sucrose caramel consists of a brownish colored substance, easily soluble in water but insoluble in strong ethyl alcohol, ether or chloroform. Caramel reduces Fehling's solution strongly; it is completely precipitated from solution by ammoniacal lead subacetate. Solutions of caramel show before the

* J. Am. Chem. Soc., **28,** 465.

† Lafar's " Technische Mykologie," Jena (1901–1907).

‡ Jörgensen's " Mikroorganismen der Gärungsindustrie," Berlin.

§ " Chemie der Zuckerarten," 1288–1317.

spectroscope characteristic absorption bands, the blue part of the spectrum being more or less extinguished according to concentration. If a caramel solution is shaken with an alcoholic solution of paraldehyde and allowed to stand in the cold for 24 hours a brownish yellow gummy precipitate will form, the rapidity of deposition depending upon the amount of caramel present. The paraldehyde-caramel compound is soluble in water from which it is reprecipitated by strong alcohol; its composition has not been definitely established.

The formation of caramel from sucrose consists primarily in the splitting off of water in successive stages, this giving rise to a series of dehydration and condensation products of varying complexity. Gelis* was the first to attempt the separation of caramel into its components and defined three different constituents, *caramelane*, *caramelene* and *carameline*. *Caramelane* was prepared by Gelis by heating sucrose until it lost about 12 per cent in weight and was given the formula $C_{12}H_{18}O_9$; *caramelene*, $C_{36}H_{48}O_{24} \cdot H_2O$, was prepared by heating sucrose until it lost about 15 per cent in weight; and *carameline*, $C_{96}H_{100}O_{50} \cdot H_2O$, by heating sucrose until it lost about 20 per cent in weight. Other investigators give caramelane, caramelene and carameline entirely different formulæ; each of these substances is probably a mixture of decomposition products so that the formulæ assigned by Gelis have a questionable value.

Caramelane was prepared by Stolle† by heating melted sucrose at 180° C. until no further loss occurred; the residue was dissolved in water, any unchanged sugar removed by fermentation and the residue evaporated in vacuo to dryness. The substance thus obtained consisted of a brownish mass melting at 134° to 136° C., its composition corresponded to the formula $C_{12}H_{18}O_9$ the same as the caramelane of Gelis.

The caramel substance *saccharan*, $C_{12}H_{18}O_9$, obtained by Ehrlich by heating sucrose to 200° C., has already been described (p. 467). It is probably identical with the caramelane of Gelis and Stolle.

**Destructive Action of Heat Upon Sucrose in Solution.** — A knowledge of the destructive changes which sucrose undergoes upon heating its aqueous solutions is of great importance. Unfortunately no fixed rule can be given for this, as the nature and extent of the decomposition depend largely upon the character of accompanying impurities.

Sucrose in perfectly neutral solutions, when heated for a few hours at 100° C., begins to undergo decomposition as a result of carameliza-

* Ann. chim. phys. [3] **52,** 352; Compt. rend., **45,** 590.

† Z. Ver. Deut. Zuckerind, **49,** 800; **51,** 836; **53,** 11–47.

tion and incipient inversion, the latter being produced according to some chemists by the H ions of dissociated molecules of water, and according to other chemists by auto-inversion, the sucrose itself behaving as an extremely weak acid. After the commencement of inversion the sucrose solution becomes perceptibly acid, and heating from this point causes decomposition and inversion to proceed with increasing rapidity.

To determine the rate of decomposition which sucrose undergoes upon heating its solutions when formation of free acid is prevented, Herzfeld* conducted experiments with solutions which were made slightly alkaline; variations in the kind and amount of alkali were not found to cause any difference in the character of the results. The following table taken from Herzfeld's work shows the percentage loss of total sucrose caused by heating solutions of different concentration at varying temperatures for 1 hour.

TABLE XCII

*Loss of Sucrose upon Heating Solutions of Different Concentration at Varying Temperatures*

| Deg. C. | 10 per cent. | 15 per cent. | 20 per cent. | 25 per cent. | 30 per cent. | 35 per cent. | 40 per cent. | 45 per cent. | 50 per cent. |
|---|---|---|---|---|---|---|---|---|---|
| 80 | 0.0444 | 0.0373 | 0.0301 | 0.0229 | 0.0157 | 0.0168 | 0.0179 | 0.0190 | 0.0200 |
| 90 | 0.0790 | 0.0667 | 0.0541 | 0.0418 | 0.0290 | 0.0317 | 0.0344 | 0.0371 | 0.0392 |
| 100 | 0.1140 | 0.0961 | 0.0781 | 0.0602 | 0.0423 | 0.0466 | 0.0508 | 0.0551 | 0.0584 |
| 110 | 0.1630 | 0.1362 | 0.1093 | 0.0825 | 0.0557 | 0.0612 | 0.0667 | 0.0721 | 0.0766 |
| 120 | 0.2823 | 0.2582 | 0.2341 | 0.2098 | 0.1857 | 0.2063 | 0.2669 | 0.2474 | 0.2678 |
| 130 | 2.0553 | 1.7582 | 1.4610 | 1.1638 | 0.8667 | 0.9451 | 1.0235 | 1.0119 | 1.1800 |
| 140 | 5.1000 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

The results show in every case a rapid increase in the destructive action between 120° and 130° C. The percentage loss is greatest with the more dilute solutions, but the absolute loss of sucrose (i. e., grams destroyed per 100 gms. solution) increases with the concentration. It should be borne in mind that the results of Table XCII show the rate of decomposition under only one set of conditions; in the absence of free alkalies the progress of decomposition would be much more rapid.

**Changes in Polarization During Heating of Sucrose Solutions.**— Prolonged heating of sucrose solutions causes a series of important changes in the polarizing power. A graphic representation of these changes is given in Fig. 198, where the ordinates represent degrees polarization and the abscissæ hours of heating.

* Z. Ver. Deut. Zuckerind, **43**, 745.

For the first few hours of heating only a slight decrease in polarization is noted, then, with the formation of acid oxidation products and the consequent increase in the rate of inversion, the polarization quickly falls until at *B* the polarization of undecomposed sucrose, and that of its inversion and decomposition products (glucose, fructose, caramel, etc.), exactly neutralize one another and the rotation is 0. Upon longer heating the remaining sucrose is inverted; the rotation of the fructose becomes the predominant factor and the polarization is levorotatory. A maximum levorotation is reached at *C*, after which, with

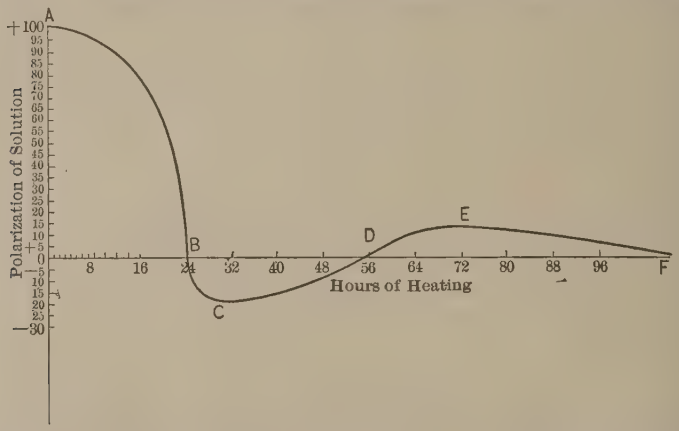



Fig. 198. — Showing changes in polarization of a sucrose solution by destructive action of heat.

the decomposition of the more unstable fructose, the rotation again approaches 0 until at *D* a second point of inactivity is reached, the rotatory powers of undecomposed fructose, glucose and other substances counterbalancing one another. Upon longer heating the remaining fructose is destroyed; the rotation of glucose is now the predominant factor and the polarization of the solution becomes dextrorotatory again. A maximum dextrorotation is reached at *E*, after which with the destruction of the glucose the polarization gradually approaches 0, until at *F* a third and final point of inactivity is reached.

The curve of changes just described may be longer or more contracted than that shown in Fig. 198 according to the temperature of heating, nature of salts and impurities present, and other conditions.

*High-polarizing Sugar.* — A condition exactly opposite to that just noted is sometimes observed in technical operations, where heating

concentrated sucrose solutions has been found under certain conditions to cause an increase in the polarization. This phenomenon has been attributed by some to the formation of high-rotating dextrinoid condensation products and by others to the splitting off of glucose in a high mutarotating form. This increase in polarization, according to Lippmann,* is observed only when the solution is neutral or very weakly acid; in presence of free alkali it does not seem to take place.

*Optically Inactive Sugar.* — If sucrose is heated with only a small amount of water at 150° to 160° C. for a short time, a mixture is obtained which shows almost complete optical inactivity. This so-called optically inactive, or neutral sugar, was first observed by Berzelius and Mitscherlich,† and has been the subject of frequent investigations since their time. Optically inactive sugar consists of a mixture of glucose and other products whose rotations neutralize one another. According to Wohl‡ the sucrose is decomposed into glucose and a condensation product of fructose which he calls levulosin.

$$\underset{\text{Sucrose}}{n\,(C_{12}H_{22}O_{11})} = \underbrace{\underset{\text{Glucose}}{n\,C_6H_{12}O_6} + \underset{\text{Levulosin.}}{(C_6H_{10}O_5)_n}}.$$

$$[\alpha]_D = +66.5 \qquad [\alpha]_D = 0.$$

Optically inactive sugar upon warming with acids becomes strongly levorotatory and this is explained by the hydrolysis of the levulosin into d-fructose.

$$\underset{\text{Levulosin}}{(C_6H_{10}O_5)_n} + n\,H_2O = \underset{\text{d-Fructose.}}{n\,C_6H_{12}O_6}.$$

## The Inversion of Sucrose

### inversion of sucrose by acids

**Early Investigations.** — One of the earliest facts noted in connection with the chemistry of sucrose was that after warming with acids the sugar could no longer be recovered in its original crystallizable form. The change was described by saying that the sucrose was converted into "uncrystallizable sugar," a term which is still occasionally used by certain writers. After the invention of the polariscope, Biot, in 1836, noted that the change which acids produced upon sucrose was attended by an alteration in the character of the rotation imparted to the plane of polarized light; the direction of rotation for the original sucrose solution was changed from right to left, or from + to −. On account of this transposition in sign the term "inversion" was applied

* "Chemie der Zuckerarten," 1223; Z. Ver. Deut. Zuckerind, **35**, 434.

† Journ. pharm. [3], **4**, 216.

‡ Ber., **23**, 2088.

to the process and the name "invert-sugar" given to the products of the reaction. It was soon observed that the sirupy sugar obtained by inverting sucrose soon crystallized with separation of glucose; Dubrunfaut,* however, was the first to explain the true character of the process and showed that the sugars glucose and fructose were both formed during inversion.

**Wilhelmy's Law of Mass Action.** — It was noted quite early in the study of inversion that the various acids differed in the rapidity of their inverting power, although the action of each acid seemed to follow one general law. The nature of this law was discovered in 1850 by Wilhelmy,† who showed that the amount of sucrose inverted by an acid in a given moment of time is always a constant percentage of the amount of unchanged sucrose present. This discovery is formulated in Wilhelmy's law of mass action; viz., the velocity of a reaction at any moment is proportional to the concentration of the reacting substance.

The inversion of sucrose by acids is expressed by the equation:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose.}}{C_6H_{12}O_6}.$$

Although this equation involves the disappearance of one molecule of water with each molecule of sucrose, and is therefore bimolecular, the diminution in the total active mass of water is so slight that the process of inversion can be treated as a unimolecular‡ reaction.

**Rate of Inversion.** — If $a$ is the original amount of sucrose present and $x$ the quantity inverted at the end of the time $t$ after the commencement of inversion, then the rate of inversion for a unimolecular reaction will be:

$$\frac{dx}{dt} = k\,(a - x), \tag{1}$$

in which $dx$ is the infinitesimal quantity of sucrose inverted during the infinitesimal period of time $dt$ and $k$ the velocity coefficient of the inversion. The constant $k$ is found by means of the integral calculus to be

$$k = \frac{1}{t} \log \text{nat.} \frac{a}{a - x}, \tag{2}$$

or, changing from natural to common logarithms,

$$(\log \text{nat.} = \log \text{com.} \div 0.4343).$$

$$k = \frac{1}{0.4343\,t} \log \text{com.} \frac{a}{a - x}. \tag{3}$$

* Compt. rend., **42,** 901; **69,** 438.

† Poggend. Ann., **81,** 413, 499.

‡ For a demonstration of this see Mellor's "Chemical Statics and Dynamics" (1909), pp. 40 and 41.

For purposes of comparison, however, formula (2) using common logarithms is often employed.

*Determination of Rate of Inversion by Polariscope.*— In applying formula (2) the polarimetric observations may be substituted for $a$ and $x$. Calling the rotation before inversion $r_0$ and after inversion $r_\infty$ and for any time $t$ during inversion $r$, then

$$k = \frac{1}{t} \log \frac{r_0 - r_\infty}{r - r_\infty}. \qquad (4)$$

The following table shows the rate of inversion at 20° C. for a normal weight (26 gms.) of sucrose made up with water and 10 c.c. of concentrated hydrochloric acid to 100 true c.c.

TABLE XCIII

*Showing Rate of Inversion of Sucrose*

| | Time. | Rotation. | $k = \frac{1}{t} \log_{10} \frac{r_0 - r_\infty}{r - r_\infty}$. |
|---|---|---|---|
| | Minutes. | Deg. V. | |
| Before inverting | 0 | +100.00 | ..... |
| | 5 | 94.05 | 0.00391 |
| | 15 | 82.80 | 0.00394 |
| | 30 | 68.20 | 0.00388 |
| | 60 | 45.40 | 0.00374 |
| | 90 | 27.40 | 0.00372 |
| | 120 | 13.80 | 0.00367 |
| | 150 | 2.80 | 0.00367 |
| | 159 | 0.00 | 0.00368 |
| | 180 | −5.85 | 0.00369 |
| | 240 | −17.35 | 0.00366 |
| | 360 | −28.95 | 0.00371 |
| Inversion complete | ∞ | −35.20 | ..... |
| | | | Aver. 0.00375 |

The results in the table show that while about 40 per cent of the total sucrose is inverted in the first hour, 24 per cent in the second hour, 14 per cent in the third hour, 9 per cent in the fourth hour, etc., yet the velocity of inversion always bears a constant ratio to the diminishing amount of sucrose present, temperature and other conditions remaining the same. Thus in the previous table 40 per cent of the total sucrose is inverted during the first hour, and during each succeeding hour always 40 per cent of the sucrose present at the beginning of the hour is inverted. It follows, therefore, since the inversion constant $k$ is the same for any concentration of sucrose that the most concentrated solutions of the latter can be completely inverted by relatively small amounts of acid.

*Errors in Polarimetric Method for Determining Rate of Inversion.* — The somewhat irregular values for the velocity coefficient, which are often obtained by the polarimetric method at the beginning of inversion, have led some investigators to suspect an exception to the law of mass action for the early stages of hydrolysis. The method of determining the rate of inversion by observing the changes in the rotation of a solution in a polariscope tube is attended with several small errors.* There is, first, the possible influence of the contraction † in volume which accompanies inversion, and which for a 25 per cent solution of sucrose is about 0.5 c.c. per 100 c.c. There is, second, the change in polarization of the liberated glucose and fructose due to mutarotation, this error, however, being greatly reduced by the accelerating influence of the acid. The supposition that the increase in concentration of fructose during inversion causes an error in the value of $k$ has been proved by Rosanoff, Clark and Sibley to be untrue, since the percentage of water in the solution remains practically constant during the inversion. Careful experiments by the above authorities, in which varying amounts of sucrose were inverted in solutions containing the same weights of acid and water per unit volume, show that the velocity coefficient is independent of the initial concentration of sucrose and is the same throughout inversion as long as the concentration of water and acid remains unchanged.

Another source of error in measuring the constant $k$ is due to the slight rise in temperature which takes place in mixing the acid and sugar solution. The speed of inversion is thus slightly accelerated at the beginning and this would explain the slightly higher values of $k$ for the first few readings of the previous table.

**Inverting Power of Different Acids.** — The inverting power of different acids has been determined by Ostwald‡ whose results are given in the following table. To avoid the use of small decimals the constant $C = 10{,}000\ k$ is employed. The second column of the table gives the relative inverting power of each acid as compared with that of hydrochloric acid which is taken as 100. In making the experiments 10 c.c. of a 40 to 50 per cent sucrose solution were inverted at 25° C. with 10 c.c. of a normal solution of the acid.

* For a fuller discussion of these errors see paper by C. S. Hudson (J. Am. Chem. Soc., **32,** 885), "Is the hydrolysis of cane sugar by acids a unimolecular reaction when observed with a polariscope?" and the paper by Rosanoff, Clark and Sibley (J. Am. Chem. Soc., **33,** 1911), "A reinvestigation of the velocity of sugar hydrolysis."

† Lippmann's "Chemie der Zuckerarten" p. 1258. ‡ J. prakt. Chem. [2], **29,** 385.

TABLE XCIV

*Showing Relative Inverting Power of Different Acids*

| Kind of acid. | Inversion constant C. | Inverting power HCl = 100. | Kind of acid. | Inversion constant C. | Inverting power HCl = 100. |
|---|---|---|---|---|---|
| Hydrobromic | 24.38 | 111.4 | Malonic | 0.674 | 3.08 |
| Benzolsulphonic | 22.82 | 104.4 | Diglycollic | 0.583 | 2.67 |
| Chloric | 22.61 | 103.5 | Methylglycollic | 0.397 | 1.82 |
| Hydrochloric | 21.87 | 100.0 | Citric | 0.377 | 1.72 |
| Nitric | 21.87 | 100.0 | Glyceric | 0.375 | 1.72 |
| Methylsulphuric | 21.86 | 100.0 | Formic | 0.335 | 1.53 |
| Isethionic | 20.07 | 91.8 | Methyllactic | 0.304 | 1.39 |
| Ethylsulphonic | 19.93 | 91.2 | Ethylglycollic | 0.300 | 1.37 |
| Trichloracetic | 16.47 | 75.4 | Glycollic | 0.286 | 1.31 |
| Sulphuric | 11.72 | 53.6 | Malic | 0.278 | 1.270 |
| Dichloracetic | 5.93 | 27.1 | Pyrotartaric | 0.234 | 1.072 |
| Oxalic | 4.00 | 18.57 | Lactic | 0.233 | 1.070 |
| Pyroracemic | 1.419 | 6.49 | Oxyisobutyric | 0.232 | 1.060 |
| Phosphoric | 1.357 | 6.21 | Succinic | 0.1192 | 0.545 |
| Monochloracetic | 1.059 | 4.84 | Acetic | 0.0876 | 0.400 |
| Arsenic | 1.052 | 4.81 | Isobutyric | 0.0733 | 0.335 |

*Relation of Inverting Power to Affinity and Electric Conductivity.* — The speed of inversion is in general proportional to the affinity and electric conductivity of the acid. This is shown in the following table, taken from the work of Ostwald, where a number of acids are arranged in order of their constants, the latter for purpose of comparison being expressed in terms of HCl = 100.

TABLE XCV

*Showing Relation of Inverting Power to Affinity and Conductivity of Acids*

| Acid. | Speed of inversion. | Chemical affinity. | Electric conductivity. |
|---|---|---|---|
| Hydrochloric | 100 | 100 | 100 |
| Nitric | 100 | 100 | 99.6 |
| Sulphuric | 53.6 | 49 | 65.1 |
| Oxalic | 18.6 | 24 | 19.7 |
| Phosphoric | 6.2 | 13 | 7.3 |
| Monochloracetic | 4.8 | 9 | 4.9 |
| Acetic | 0.4 | 3 | 0.4 |

The order of magnitude of the constants for the different acids is the same. This parallelism is explained by the dissociation theory of Arrhenius as due to the fact that the inverting power, affinity and conductivity of acids are dependent upon their degree of ionization, or, in other words, upon the relative amounts of hydrogen ions in

solution. The formula for the inversion of sucrose is in fact sometimes written:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} + \underset{\text{H ion}}{\overset{+}{H}} = 2\,\underset{\text{Invert sugar}}{C_6H_{12}O_6} + \underset{\text{H ion,}}{\overset{+}{H}},$$

one $\overset{+}{H}$ ion participating in an unlimited number of reactions. Many hypotheses have been proposed to account for the catalytic action performed by the $\overset{+}{H}$ during the inversion of sucrose, such as vibratory action, carrier of water, etc., but no satisfactory explanation has as yet been found.

*Influence of Temperature Upon Speed of Inversion.* — Elevation of temperature produces a marked increase in the inverting power of acids, the velocity coefficient $k$ increasing about 15 per cent for each 1° C. elevation. This rate of increase, which is approximately the same for all acids, diminishes, however, with rise in temperature; the total increase in $k$ from 0° to 10° C. was found by Hammerschmidt* to be about 500 per cent, from 30° to 40° C. about 400 per cent and from 70° to 80° C. about 300 per cent.

*Arrhenius's Hypothesis of "Active" and "Inactive" Sucrose Molecules.* —Inasmuch as the ionization of acids in aqueous solution is not greatly affected by changes in temperature and as the coefficient for the increase in speed of the $\overset{+}{H}$ ions for 1° C. increase is only a small percentage of the increase observed for the inversion constant $k$, Arrhenius† adopted the hypothesis that solutions of sucrose contained "active" and "inactive" molecules, the amount of "active sucrose" being relatively small, as compared with the "inactive," but this amount increasing, at the expense of the "inactive" sucrose, by about 12 per cent for each 1° C. increase. This transformation of "inactive" into "active" sucrose precedes inversion and is supposed to take place through addition of water or by some process of molecular rearrangement. Upon this hypothesis Arrhenius has derived the following formula for expressing the influence of temperature upon the inversion of sucrose between 0° and 55° C.:

$$Ct_1 = Ct_0 e^{\frac{q}{2}\left(\frac{T_1 - T_0}{T_1 T_0}\right)},$$

in which $Ct_1$ and $Ct_0$ are the inversion coefficients of the acid at the temperatures $t_1$ and $t_0$, $T_1$ and $T_0$ being the corresponding temperatures in absolute degrees; $e$ is the constant 2.71828 (the natural logarithmic base) and $q$ is the thermal constant for the transformation of "inactive" into "active" sucrose which is estimated to be 25,600 calories

* Z. Ver. Deut. Zuckerind, **40**, 408.

† Z. physik. Chem., **4**, 227.

per gram molecule of "inactive" sucrose. This formula of Arrhenius according to Ley* also holds for temperatures above 55° C.

*Hypothesis of Sucrose Ions.* — Of other hypotheses, which have been proposed to explain the effect of temperature upon inversion velocity, may be mentioned the so-called "acid nature" of sucrose in accordance with which sucrose is supposed to become dissociated into ions. The formation of saccharates or salts of sucrose is used as one argument for this hypothesis; solutions of sucrose, however, show perfect neutrality to the most sensitive indicators, and are absolute non-conductors of electricity, so that no direct evidence exists to support the hypothesis of sucrose H ions.

*Influence of Concentration and of Salts Upon Inverting Power of Acids.* — The inversion velocity of sucrose by means of acids is in general proportionate to the concentration of H ions; strict conformity to this rule, however, obtains only with pure dilute solutions of the acid. The proportionality of the inversion constant $k$ to concentration of H ions shows marked deviations at high concentrations of acid or in presence of neutral salts. Thus the proportionality in H ion concentration of 0.1 normal to 0.5 normal nitric acid is not 1: 5 but 1: 4.64; the proportionality in inverting power, however, is 1: 6.07. This increase in the proportionality of the inversion constant is explained by an increase in the *speed* of the H ions. In the same way addition of potassium nitrate to nitric acid will lower the concentration of H ions, but cause an increase in inversion velocity, this increase being explained by the increase in speed imparted by the dissociated molecules of potassium nitrate to the remaining H ions.

The observations just noted for nitric acid and potassium nitrate hold, however, only for the strong acids and their corresponding neutral salts. With weak acids an exactly opposite effect is noted. Increasing the concentration of acetic acid, for example, lowers the proportionality of the inversion constant $k$; so also the addition of an equivalent amount of potassium acetate to acetic acid will reduce the value of $k$ to $\frac{1}{10}$ of its original amount.

Additions of neutral salts of a different acid than the inverting agent produce variable effects. Thus sodium sulphate diminishes while sodium chloride increases the inversion velocity of acetic acid.

In addition to the view that neutral salts alter the activity of the H ions, Arrhenius supposes that the amount of "active sucrose" is also affected, while other chemists hold that the molecules of water undergo dissociation to a greater or less degree.

* Z. physik. Chem., **30**, 253.

Organic non-conductors, such as alcohol, acetone, etc., if present in large amounts, diminish the inversion velocity of acids to a marked degree, although the electric conductivity of the solution itself may not be appreciably lessened. In such cases it is supposed that the movement of the H ions is in some way retarded.

Further discussion of the numerous hypotheses which have been proposed in this connection must be passed over; for a fuller treatment of the inversion of sucrose by acids and the relationship of the subject to the dissociation theory the student is referred to Lippmann,* or to the more special treatises upon physical chemistry.

### INVERSION OF SUCROSE BY SALTS

Sucrose is inverted upon heating with solutions of metallic salts; the speed of inversion, as in the case of acids, is in general proportionate to the concentration of hydrogen ions, the latter being formed by a hydrolysis of the salt in presence of water according to the following equation:

$$\underset{\text{Salt}}{MA} + \underset{\text{Water}}{HOH} = \underset{\text{Hydroxide}}{MOH} + \underset{\text{Dissociated acid}}{\overset{+}{H} \cdot A},$$

in which M is the metal and A the acid radical. The concentration of H ions, and hence the speed of inversion, depends upon the extent of hydrolysis and dissociation.

A number of investigators have studied the inversion of sucrose by salts. Walker and Aston,† working with sucrose solutions at 80° C., found the following inversion constants for a number of nitrates:

| | |
|---|---|
| Cadmium nitrate ($N/2$) | 0.000154 |
| Zinc nitrate ($N/2$) | 0.000207 |
| Lead nitrate ($N/2$) | 0.001590 |
| Aluminum nitrate ($N/2$) | 0.007700 |

The same order, Cd, Zn, Pb and Al, has also been found by other investigators. Long,‡ who has made an extensive study of the inverting action of salts, found for several sulphates the inversion to increase in the order Mn, Zn, Fe and Al. Kahlenberg, Davis and Fowler§ from a study of the inverting power of different salts at 55.5° C. (the temperature of boiling acetone) by the polariscopic and freezing-point methods obtained the following results:

* "Chemie der Zuckerarten," 1257–1303.
† J. Chem. Soc., **67**, 576.
‡ J. Am. Chem. Soc., **18**, 120, 693.
§ J. Am. Chem. Soc., **21**, 1.

| Salt. | Concentration. (Gram molecules per 1000 c.c.) | | Method. | $k$. |
|---|---|---|---|---|
| | Salt. | Sucrose. | | |
| Manganese sulphate | $\frac{1}{4}$ | $\frac{1}{2}$ | Polariscope | 0.0000 |
| Manganese chloride | $\frac{1}{4}$ | $\frac{1}{2}$ | Polariscope | 0.000163 |
| Cadmium chloride | $\frac{1}{4}$ | $\frac{1}{2}$ | Polariscope | 0.0014* |
| Nickel sulphate | $\frac{1}{4}$ | $\frac{1}{2}$ | Freezing point | 0.0028* |
| Copper sulphate | $\frac{1}{4}$ | $\frac{1}{2}$ | Polariscope | 0.0055* |
| Copper chloride | $\frac{1}{4}$ | $\frac{1}{2}$ | Freezing point | 0.0069* |
| Mercuric chloride | $\frac{1}{8}$ | $\frac{1}{2}$ | Polariscope | 0.0057 |
| Mercuric chloride | $\frac{1}{8}$ | $\frac{1}{2}$ | Freezing point | 0.0054 |
| Aluminum sulphate | $\frac{1}{12}$ | $\frac{1}{2}$ | Polariscope | 0.0303 |
| Aluminum chloride | $\frac{1}{6}$ | $\frac{1}{2}$ | Polariscope | 0.0422 |

In the results marked with a * the values of $k$ were not found to run constant during the experiment, so that the figures represent only a rough average.

As a general rule it may be stated that the inverting power of neutral salts of the same acid follows approximately the basicity or position of the metal in the electro-chemical series, i.e., increasing in the order: K, Na, Ba, Sr, Ca, Mg, Al, Mn, Zn, Cd, Fe, Co, Ni, Sn, Pb, Cu, Bi, Sb and Hg. Important exceptions to this rule occur, however, as in the case of aluminum, the salts of which, notwithstanding its high position in the electro-chemical series, have a higher inversion coefficient than any of the metals thus far studied. The inverting power of neutral salts of the same base increases in general with the strength or position of the acid in the electro-chemical series, i.e., increasing in the order: acetic, tartaric, oxalic, sulphuric, nitric, hydrochloric, etc. Chlorides, for example, invert sucrose faster than sulphates of the same metal, since they are more easily dissociated and hence produce a greater concentration of H ions.

The salts of the weakest bases and strongest acids have, therefore, in general the most powerful inverting action.

*Influence of Invert Sugar Upon the Inverting Power of Salts.* — Of great importance in this connection is the marked increase in the inverting power of neutral salts produced by the presence of reducing sugars. Prinsen Geerligs* has made a special study of this question, and the following is taken from the results of his investigations.

The increase in inverting power of salts produced by the presence of invert sugar is shown in the following series of experiments where 50 c.c. of solutions containing 50 per cent sucrose, 1 gm. sodium

* Deut. Zuckerind, **23**, 292.

chloride, and 5, 10, 20 and 30 per cent invert sugar were heated to 100° for 3 hours.

| Per cent invert sugar | 5 | 10 | 20 | 30 |
|---|---|---|---|---|
| Per cent sucrose inverted | 7.47 | 15.05 | 21.93 | 27.50 |

The influence of different salts of the same acid is shown in the following series, where 50 c.c. of solutions containing 40 per cent sucrose and 25 per cent invert sugar were heated at 100° C. for 2 hours with a quantity of different chlorides equivalent to 1.75 gm. Cl.

| Salt | KCl | NaCl | LiCl | $CaCl_2$ | $SrCl_2$ | $BaCl_2$ | $MgCl_2$ |
|---|---|---|---|---|---|---|---|
| Per cent sucrose inverted | 33.80 | 35.46 | 39.68 | 40.65 | 47.60 | 50.01 | 50.01 |

The influence of different salts of the same base is shown in the following series, where 50 c.c. of solutions containing 40 per cent sucrose and 10 per cent invert sugar were heated at 100° C. for 2 hours with a quantity of different potassium salts equivalent to 1.75 gms. Cl in KCl.

| Salt | K-acetate | K-tartrate | K-oxalate | $KClO_3$ | $K_2SO_4$ | $KNO_3$ | KI | KBr | KCl |
|---|---|---|---|---|---|---|---|---|---|
| Per cent sucrose inverted | 0.00 | 0.07 | 0.30 | 3.32 | 3.80 | 4.94 | 4.94 | 6.27 | 6.27 |

The inverting power of the different salts is seen to follow the positions of the metal and acid in the electro-chemical series, the salts of the weakest bases and strongest acids having the highest power of inversion.

The substitution of other reducing sugars was found by Geerligs to produce the same effect as glucose and fructose in increasing the inverting power of neutral salts. Non-reducing sugars, such as raffinose, had no sensible action.

The action of reducing sugars in increasing the inverting power of salts has been explained by the formation of basic sugar compounds, the hydrolysis of the salt and formation of H ions being thus facilitated.

$$\underset{\text{Salt}}{MA} + \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Water}}{HOH} = \underset{\text{Basic glucose compound}}{MOH \cdot C_6H_{12}O_6} + \underset{\text{Ionized acid.}}{\overset{+}{H} \cdot \overset{-}{A}}.$$

Deerr,* who has recently made a study of the question, concludes that the combined influence of glucose and neutral salts does not produce inversion. This conclusion, which is exactly opposite to that of Geerligs, leaves the subject open to further investigation.

The inverting power, which different salts may have upon sucrose, under the varying conditions of manufacture and analysis, is a factor which the chemist must always bear in mind.

### INVERSION OF SUCROSE BY INVERTASE

**Occurrence of Invertase.** — The most important inverting agent of sucrose from a physiological point of view is *invertase.* This enzyme is found widely distributed in the vegetable and animal kingdom, being

* Bull. 35, Hawaiian Sugar Planters' Experiment Station.

secreted by all living cells where sucrose undergoes metabolism. Invertase occurs in many bacteria, in nearly all yeasts, in different moulds, as *Aspergillus* and *Penicillium*, and in the leaves, buds, fruit, reserve organs and other tissues of many higher plants, where sucrose is utilized either for the building up of new tissue or for transportation to points of growth.

In the animal kingdom invertase is found in the intestinal juice and other fluids of the body. Extracts prepared from the mucous membrane of the intestines, from the kidneys and other organs are strongly inverting. Invertase is also found in the digestive tract of many insects; its presence in the honey sac of the bee has already been referred to. While the invertases from different sources resemble one another in their hydrolytic action upon sucrose, they show certain differences in behavior. It is supposed, therefore, that the inverting enzymes constitute a group, the different members of which are not strictly identical. On account of the difficulty of preparing perfectly pure preparations of invertase, it has been impossible to determine the identity or difference of the enzyme from the various plant and animal sources.

**Preparation of Invertase.** — Invertase is best obtained from yeast, and various methods have been devised for preparing the enzyme from this source. Some authorities recommend mixing fresh washed yeast with powdered glass or sand and air drying. The mass is then ground in a mill or mortar and extracted with cold water using a powerful press to increase the extraction.

A more active preparation of invertase than that obtained by the above process is obtained by the method of O'Sullivan and Tompson* in which yeast is subjected to autolytic digestion. Pure fresh brewer's yeast is washed, drained and then set aside in a covered jar for several weeks at ordinary temperature until the mass has liquefied. A dark yellow solution is obtained which can be purified and decolorized by filtering through bone black. The autolysis may be hastened by first destroying the life of the yeast cell with chloroform as recommended by Fischer.†

The method of Hudson‡ for preparing a stock solution of invertase is as follows: "Break up 5 pounds of pressed yeast, which may be either baker's or brewer's yeast, add 30 c.c. of chloroform to it in a closed flask and allow it to stand at room temperature (20° C.) over night. By the morning, the solid mass will have become fluid and it

* J. Chem. Soc., **57**, 834–931. † Ber., **27**, 2985.
‡ J. Ind. Eng. Chem., **2**, 143.

should then be filtered through filter paper, allowing several hours for draining. To the filtrate add neutral lead acetate until no further precipitate forms and again filter. Precipitate the excess of lead from the filtrate with potassium oxalate and filter. To this filtrate add 25 c.c. of toluene and dialyze the mixture in a pig's bladder for 2 or 3 days against running tap water. The dialyzed solution is colorless, perfectly clear after filtration, neutral to litmus, has a solid content of about one-half of one per cent, an ash content of a few hundredths of one per cent, will keep indefinitely in an ice box if a little toluene is kept on its surface to prevent the growth of microörganisms, and is exceedingly active in inverting cane sugar. The invertase solution does not reduce Fehling's solution." The solution of invertase prepared by this method gives a dextrorotation of 1° V. in a 400-mm. tube.

Invertase is precipitated from solution by adding about 3 vols. of strong alcohol. The precipitate is filtered off, and finally dried in a vacuum over concentrated sulphuric acid. The product can be purified by redissolving in water and again precipitating by means of alcohol; such purification, however, is always attended by loss in inverting power.

**Properties of Invertase.** — Dry invertase consists of a white powdery substance easily soluble in water with formation of a yellowish neutral solution. Unless previously dialyzed the product contains considerable mineral matter, the quantity sometimes exceeding 20 per cent. The chemical composition of invertase is not fully known. Barth* found for an ash-free preparation 43.9 per cent C, 8.40 per cent H, 6.00 per cent N and 0.63 per cent S. Osborne† found 44.54 per cent C, 6.52 per cent H and 6.1 per cent N. The high percentage of nitrogen, the positive reaction with Millon's reagent and the biuret test indicate the presence of an albuminoid group. Carbohydrates, consisting probably of mannan and pentosan groups, have also been found in invertase. It is uncertain whether these carbohydrate groups are a constituent part of the enzyme or like the mineral matter consist only of accompanying impurities.

**Conditions Affecting the Activity of Invertase.** — The inversion of sucrose by invertase consists in the addition of one molecule of water to each molecule of sugar, but the mechanism of this process is not as yet understood. It is supposed by some that the configuration of the enzyme must conform in certain respects to that of the sugar hydrolyzed and this is used as an argument for the presence of a carbohydrate group in invertase. Fischer has likened the relation of enzyme to

* Ber., **11**, 474. † Chem. News, **79**, 277.

sugar to that existing between a key and lock, the shape of the key permitting it to unfasten only that lock to whose structure it corresponds. The action of invertase being purely catalytic, a small amount of enzyme can invert almost unlimited quantities of sucrose. O'Sullivan and Tompson found in fact that a preparation of invertase, which had already inverted 100,000 parts of sucrose, had lost none of its activity.

*Influence of Acids and Alkalies on Activity of Invertase.* — To secure the maximum inverting power invertase must be allowed to act in a weakly acid solution. The acidity for acids, which are largely dissociated as hydrochloric acid, should not greatly exceed $n/1000$. An acidity much above $n/100$ HCl will completely destroy invertase. For acids which are only slightly dissociated, as acetic, the acidity may exceed 100 times the concentration permissible for hydrochloric acid.

In analytical work it is best to use invertase in an acetic acid solution; an acetic acidity just sufficient to redden litmus was found by Hudson* to give the best results.

Invertase is rendered completely inactive by small amounts of alkali; in such cases the original activity may be regained by restoring the proper degree of acidity. Addition of alkalies in large amount destroys the enzyme completely.

*Rate of Inversion by Invertase.* — The inversion velocity of sucrose by means of invertase has been a subject of considerable study and the conclusion of early observers has been that the inversion does not follow the formula for a unimolecular reaction, such as is obtained by inversion with acids. O'Sullivan and Tompson,† however, showed, in 1890, that in following the inversion with invertase a serious error existed in the polarimetric reading if the mutarotation of the freshly liberated sugar was not considered. To quote from these authors:

"The dextrose formed by the action of invertase on cane sugar is initially in the birotary state, and, therefore, the optical activity of a solution undergoing inversion is no guide to the amount of inversion that has taken place. If a caustic alkali be added to a solution undergoing inversion, and the optical activity be allowed sufficient time to become constant, it is a true indicator of the amount of inversion that had taken place at the moment of adding the alkali."

When the error due to mutarotation is thus corrected, the inversion by invertase was found by O'Sullivan and Tompson to follow the same unimolecular formula as by inversion with acids.

The action of invertase upon sucrose has recently been studied by

* J. Ind. Eng. Chem., **2**, 143.

† J. Chem. Soc., **57**, 927.

Hudson * and the conclusions of O'Sullivan and Tompson were fully confirmed. Hudson, for example, found for the apparent and real rate of inversion by invertase the following values:

TABLE XCVI

*Apparent and Real Rates of Inversion of Sucrose by Invertase*

| Time ($t$). | Rotation. | | $k = \frac{1}{t} \log \frac{r_0 - r_\infty}{r - r_\infty}$. | |
|---|---|---|---|---|
| | Without alkali (apparent rate). | With alkali (real rate). | Without alkali. | With alkali. |
| 0 | 24.50 | 24.50 | ..... | ..... |
| 30 | 16.85 | 14.27 | 0.00396 | 0.00558 |
| 60 | 10.95 | 7.90 | 0.00399 | 0.00530 |
| 90 | 4.75 | 3.00 | 0.00464 | 0.00539 |
| 110 | 1.95 | 0.80 | 0.00482 | 0.00534 |
| 130 | −0.55 | −1.49 | 0.00511 | 0.00559 |
| 150 | −2.20 | −2.40 | 0.00522 | 0.00533 |
| ∞ | −7.47 | −7.47 | ..... | ..... |

The values of $k$ without alkali show an apparently increasing inversion velocity, a circumstance which led the early investigators to conclude that the rate of inversion with invertase did not follow the same law as for acid inversion. The value of $k$, after destroying mutarotation with a little sodium carbonate, is, however, constant within the limits of experimental error and shows that the inversion with invertase follows the law of a unimolecular reaction.

*Hudson's Equation for Inversion.* — The inversion of sucrose is represented by Hudson as follows:

$$\text{Sucrose} \begin{cases} \nearrow \alpha\text{-glucose} \rightleftarrows \beta\text{-glucose} \\ \searrow \alpha\text{-fructose} \rightleftarrows \beta\text{-fructose}. \end{cases}$$

The freshly liberated glucose and fructose are in the mutarotating form. With acid inversion the mutarotations are so accelerated that the errors in polarimetric observation largely disappear; with invertase inversion, however, the mutarotations are not accelerated and, unless destroyed with alkali, follow the ordinary rate of mutarotation for aqueous solutions, which, according to the determinations of Osaka (p. 187), is about 10 times as fast for fructose as for glucose.

Hudson has studied the mutarotation, which follows the nearly instantaneous inversion of sucrose with strong invertase at 0° C., and concludes that the freshly liberated or $\alpha$-glucose has a specific rotation

* J. Am. Chem. Soc., **30,** 1160, 1564; **31,** 655; **32,** 985, 1220, 1350.

of about + 109 and the freshly liberated or $\alpha$-fructose a rotation of about + 17, the combination of these values, when the $\alpha$-glucose and $\alpha$-fructose are molecularly united, giving the specific rotation of sucrose, i.e.,

$$[\alpha]_D \text{ sucrose} = \frac{(109 \times 180) + (17 \times 180)}{342}.$$

*Influence of Concentration of Invertase on Rate of Inversion.* — The velocity of inversion with invertase was found by O'Sullivan and Tompson to be proportional to the concentration of enzyme. This proportionality was tested by Hudson and found to be true for sucrose solutions of varying concentration. The following table by Hudson shows the percentage inversion of three sucrose solutions using different concentrations of invertase for different periods of time. In making the experiments small quantities of invertase solution were diluted to $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$, and 10 c.c. of these dilutions added to 100 c.c. of stock solutions of sucrose, the concentration of the resulting solutions being 45.5 gms., 90.9 gms. and 273 gms. sucrose per liter.

TABLE XCVII

*Influence of Concentration of Invertase on the Rate of Inversion at 30° C.*

| Concentration of invertase. | Time of action. | Concentration, × time. | Per cent inversion. | | |
|---|---|---|---|---|---|
| | | | 45.5 gms. per liter. | 90.9 gms. per liter. | 273 gms. per liter. |
| | Minutes | | | | |
| 2.00 | 15 | 30 | 73.2 | 45.3 | 11.2 |
| 2.00 | 30 | 60 | 93.0 | 74.2 | 22.0 |
| 1.50 | 20 | 30 | 73.2 | 44.8 | 11.2 |
| 1.50 | 40 | 60 | 92.8 | 74.5 | 22.7 |
| 1.00 | 30 | 30 | 72.9 | 45.3 | 11.5 |
| 1.00 | 60 | 60 | 93.0 | 74.7 | 22.3 |
| 0.50 | 60 | 30 | 72.9 | 45.2 | 11.4 |
| 0.50 | 120 | 60 | 92.7 | 74.5 | 22.6 |
| 0.25 | 120 | 30 | 73.1 | 45.2 | 10.9 |
| 0.25 | 240 | 60 | 92.7 | 74.7 | 21.9 |

The solutions of the same sucrose concentration show the same extent of inversion when the product of invertase concentration and time of action is the same. In other words the times are inversely proportional to the concentrations of invertase, from which it follows that the velocity of inversion is directly proportional to the concentration of invertase.

*Influence of Concentration of Sucrose on Activity of Invertase.* — The activity of invertase is greatly influenced by the concentration of sucrose. This is shown in the preceding table by Hudson from which the following figures are taken:

| Concentration of sucrose per 100 c.c. | I. | II. | III. |
|---|---|---|---|
| | 4.55 gms. | 9.09 gms. | 27.3 gms. |
| Per cent sucrose inverted in 15 minutes .. | 73.2 | 45.3 | 11.2 |
| Per cent sucrose inverted in 30 minutes .. | 93.0 | 74.2 | 22.0 |
| Grams sucrose inverted in 15 minutes.... | 3.32 | 4.12 | 3.06 |
| Grams sucrose inverted in 30 minutes.... | 4.23 | 6.74 | 6.01 |

It will be seen that the percentage inversion is greater the more dilute the sucrose solution. This is not true, however, as regards the absolute weight of sucrose inverted which is greatest for the solution of 9.09 gms. concentration. In 50 per cent sucrose solutions the activity of invertase at ordinary temperature is almost suspended and in saturated sucrose solutions is completely so.

*Influence of Temperature on Activity of Invertase.* — The activity of invertase is intensified by increase in temperature up to the point where the enzyme begins to undergo destruction. The optimum temperature for the maximum action of invertase is generally placed at about 55° C., although variations in concentration of sugar, changes in acidity of solution, presence of alcohol and other substances may raise or lower this figure considerably.

Perfectly dry invertase may be heated to 100° C. and even to 160° C. without losing its activity.* In presence of water, however, the enzyme is much more susceptible to the action of heat. Hudson and Paine† found that the rate of destruction by acids and alkalies increased as the temperature rose above 0° C. At about 60° C. distilled water begins to destroy the enzyme, this destruction becoming very rapid at 65° C.

*Influence of Alcohol on Activity of Invertase.* — Alcohol was found by O'Sullivan and Tompson to lessen the activity of invertase very strongly, 5 per cent of alcohol diminishing the velocity constant by nearly 50 per cent. Hudson and Paine found that above 20 per cent alcohol the inactivation was attended by a destruction of the enzyme; the rate of destruction for alcohol of different concentrations is given in the following table:

* Salkowski, Z. physiol. Chem., **31,** 304. † J. Am. Chem. Soc., **32,** 985.

TABLE XCVIII

*Rate of Destruction of Invertase by Alcohol*

| Concentration of alcohol (volume per cent) | Rate of destruction. | Concentration of alcohol (volume per cent). | Rate of destruction. |
|---|---|---|---|
| 0 | 0 | 50 | 850 |
| 10 | 0 | 55 | 570 |
| 20 | 3 | 60 | 240 |
| 30 | 44 | 70 | 74 |
| 40 | 260 | 80 | 7 |
| 45 | 487 | 90 | 2 |

It is seen that the rate of destruction attains its maximum at about 50 per cent alcohol; addition of alcohol beyond 50 per cent begins to precipitate the invertase, and this no doubt protects the enzyme as is shown by the rate of destruction falling nearly to 0 at 90 per cent alcohol.

The rate of destruction of invertase by alcohol, acids, alkalies and hot water was found by Hudson and Paine to follow the course of a unimolecular reaction.

TABLE XCIX

*The Action of Fructose in Protecting Invertase from Destruction by Acids, Alkalies, and Hot Water*

| Temperature. | Concentration of destroying agent. | Concentration of fructose. | Rate of destruction. |
|---|---|---|---|
| Deg. C. | | | |
| 30 | 0.04 normal HCl | 0.0 | 100 |
| | | 2.7 | 26 |
| | | 5.4 | 12 |
| | | 10.9 | 2 |
| 30 | 0.03 normal NaOH | 0.0 | 100 |
| | | 2.7 | 3 |
| | | 5.4 | 3 |
| | | 10.9 | 4 |
| 30 | 50 per cent alcohol | 0.0 | 100 |
| | | 2.7 | 1 |
| | | 5.4 | 1 |
| | | 10.9 | 1 |
| 61 | Distilled water | 0.0 | 100 |
| | | 2.7 | 32 |
| | | 5.4 | 16 |
| | | 10.9 | 24 |

*Protective Action of Sucrose and Fructose Upon Invertase.* — An important fact to be noted in this connection is the protective action which sucrose and fructose have in retarding the destruction of invertase. Kjeldahl,* O'Sullivan and Tompson, Hudson and Paine and

* Lippmann's " Chemie der Zuckerarten," p. 1297.

others, who have investigated this phenomenon, show that in presence of sucrose invertase can withstand higher temperatures and higher concentrations of alcohol than where no sucrose is present. The action of fructose in protecting invertase from destruction by acids, alkalies and hot water is shown in Table XCIX by Hudson and Paine* where the rates of destruction are expressed as per cent of the rate for the destroying agent when no fructose is present.

The property which sugars have of protecting invertase from destruction has been noted in case of other enzymes (as diastase); the phenomenon can be explained by assuming that the invertase forms a combination with the sugar which is less easily destroyed than the pure enzyme.

### COMPOUNDS OF SUCROSE

Owing to the absence of free aldehyde or ketone groups sucrose does not form hydrazones, osazones, oximes or other compounds such as are characteristic of the reducing sugars. Acetic anhydride under varying conditions gives a number of acetates, and benzoyl chloride in presence of sodium hydroxide gives several benzoates of sucrose. These compounds have, however, but little importance and their description is passed over.

The most important compounds of sucrose from the analytical and technical standpoint are the saccharates, or sucrates, which are formed by the combination of sucrose with various metallic bases.

**Saccharates of the Alkalies.** — By treating alcoholic sucrose solutions with concentrated potassium or sodium hydroxide, gelatinous saccharates are precipitated of the formulæ $C_{12}H_{21}KO_{11}$ and $C_{12}H_{21}NaO_{11}$. The compounds are soluble in water and dilute alcohol, but insoluble in strong alcohol. The alkali monosaccharates are also formed in aqueous solutions of sucrose after addition of potassium or sodium hydroxide, even in slight amounts. Dubrunfaut in fact noted that after addition of sodium hydroxide to sucrose in equal molecular proportions the specific rotation sank to a fixed value, further addition of alkali producing no change. The specific rotation of sodium saccharate according to Thomsen† follows the equation:

$$[\alpha]_D = + 56.84 + 0.011359\, q + 0.00039944\, q^2,$$

in which $q$ is the per cent water in solution. The depressing influence of sodium hydroxide and potassium hydroxide upon the rotation of sucrose, through formation of saccharates, may introduce an error in

* J. Am. Chem. Soc., **32**, 988.

† Ber., **14**, 1647.

certain polarimetric measurements unless the free alkali is first neutralized (preferably by means of acetic acid).

**Saccharates of the Alkaline Earths.** — The most important saccharates from the technical standpoint are those of the alkaline earths. In the formation of these the sucrose molecule can combine with one or more molecules of the base. In case of calcium there are three well characterized sucrose compounds the mono-, bi- and trisaccharates; tetra-, hexa- and octosaccharates have also been described. The structural constitution of these and other saccharates is not as yet understood, the place and manner of attachment of the base to the sucrose molecule not having been established. It is supposed that the bivalent metals are attached to the sucrose molecule by only one valency, as, for example, $C_{12}H_{21}O_{11} - Ca - OH$ in calcium monosaccharate. The existence of sucro-carbonates in which the bivalent metal is united both with sucrose and the carbonic acid radical is explained upon this supposition.

*Calcium monosaccharate* is formed by dissolving sucrose and fresh finely powdered quick lime in equal molecular proportions in water at low temperature. The compound is then precipitated from solution by strong alcohol; as thus prepared it has the formula:

$$C_{12}H_{22}O_{11} \cdot CaO + 2\,H_2O,$$

the water of crystallization being expelled by drying at 100° C. Calcium monosaccharate consists of a white amorphous substance, easily soluble in cold water but insoluble in strong alcohol; its aqueous solutions upon warming become turbid, but the turbidity disappears on recooling. Upon heating its solutions calcium monosaccharate is decomposed into calcium trisaccharate and free sucrose.

$$\underset{\text{Calcium monosaccharate}}{3\,C_{12}H_{22}O_{11} \cdot CaO} = \underset{\text{Calcium trisaccharate}}{C_{12}H_{22}O_{11} \cdot 3\,CaO} + \underset{\text{Sucrose.}}{2\,C_{12}H_{22}O_{11}}.$$

*Calcium bisaccharate* is best prepared, according to Lippmann,* by adding fresh finely powdered quick lime, free from hydroxide, to a cold aqueous solution of sucrose using 2 molecular parts of CaO to 1 of $C_{12}H_{22}O_{11}$. Upon cooling the solution with ice beautiful white crystals will separate of the composition $C_{12}H_{22}O_{11} \cdot 2\,CaO$. Crystallization at higher temperatures takes place with difficulty, and the bisaccharate, which is then obtained, contains water of crystallization. Calcium bisaccharate is soluble in about 33 parts of cold water; upon boiling the solution it is decomposed into the trisaccharate and free sucrose.

$$\underset{\text{Calcium bisaccharate}}{3\,C_{12}H_{22}O_{11} \cdot 2\,CaO} = \underset{\text{Calcium trisaccharate}}{2\,C_{12}H_{22}O_{11} \cdot 3\,CaO} + \underset{\text{Sucrose.}}{C_{12}H_{22}O_{11}}.$$

* Z. Ver. Deut. Zuckerind, **33,** 883.

*Calcium trisaccharate* is formed upon boiling solutions of the mono- and bisaccharate as above described. It is also produced as a granular precipitate by adding fresh finely pulverized quick lime to an alcoholic solution of sucrose using 3 molecular parts of CaO to 1 of $C_{12}H_{22}O_{11}$; the compound thus obtained, after drying over concentrated sulphuric acid, has the formula $C_{12}H_{22}O_{11} \cdot 3\,CaO + 4\,H_2O$, one molecule of water, however, being given off in vacuo. The trisaccharate as prepared from hot aqueous solutions has 3 molecules of water. Calcium trisaccharate is a white granular compound, soluble in 100 parts of cold and in 200 parts of hot water.

Calcium trisaccharate is employed technically in the separation of sucrose from beet molasses. In the old *elution* process of Scheibler* the molasses was mixed with an excess of freshly burned, finely powdered quick lime, and the porous mass of saccharate thus obtained freed from impurities by washing with dilute alcohol. The elution method is supplanted at present by the trisaccharate process of Steffens* which is carried out as follows. The molasses after diluting to 12 to 14 Brix is treated in the cold with freshly burned quick lime, reduced to the fineness of dust, in the ratio of 80 to 150 parts by weight of CaO to 100 of sucrose. Constant agitation of the solution is necessary in order to secure proper distribution of the lime and to prevent too great an increase in temperature. The granular precipitate of trisaccharate is filtered cold through filter presses, washed with cold water and then either used for saturating the diffusion juice, or worked up separately for sucrose by decomposing with carbon dioxide in aqueous suspension.

$$\underset{\text{Calcium trisaccharate}}{C_{12}H_{22}O_{11} \cdot 3\,CaO} + \underset{\text{Carbon dioxide}}{3\,CO_2} = \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{Calcium carbonate.}}{3\,CaCO_3}.$$

Double saccharates, in which one molecule of CaO in the trisaccharate is replaced by $K_2O$ or $Na_2O$, have also been formed. Sucro-carbonates have also been prepared; the exact nature of the latter, to which such formulæ as $C_{12}H_{22}O_{11} \cdot 6\,CaO \cdot 3\,CO_2$ have been given, is unknown.

*Strontium monosaccharate* is best obtained according to Scheibler† by treating a 20 to 25 per cent solution of sucrose at 70° to 75° C. with equal molecular parts of crystallized strontium hydroxide ($Sr(OH)_2 + 8\,H_2O$) and allowing the supersaturated solution to cool with exclusion of the carbon dioxide of the air. By adding a few crystals of monosaccharate from another preparation and agitating the solution, strontium mono-

* For a very complete description of the osmose, elution, strontia and other processes for desaccharifying molasses see Ware's "Beet Sugar Manufacture and Refining" (1907), Vol. II, 466–510, or the works of Claassen, Newlands, Rümpler, Stohmann and others.

† Ber., **16**, 984.

saccharate will separate out in cauliflower-like masses of white crystals with a composition corresponding to the formula $C_{12}H_{22}O_{11} \cdot SrO + 5\ H_2O$. The compound dissolves in warm water with great readiness to form supersaturated solutions, which may be cooled again without separation of crystals. Upon heating its solutions above 60° C. strontium monosaccharate is decomposed into bisaccharate and free sucrose.

$$\underset{\text{Strontium monosaccharate}}{2\ C_{12}H_{22}O_{11} \cdot SrO} = \underset{\text{Strontium bisaccharate}}{C_{12}H_{22}O_{11} \cdot 2\ SrO} + \underset{\text{Sucrose.}}{C_{12}H_{22}O_{11}}.$$

*Strontium bisaccharate* is best prepared according to Scheibler* by dissolving crystallized strontium hydroxide in a boiling 15 per cent sucrose solution. As soon as the molecular proportion of strontium to sucrose exceeds 2 : 1 the bisaccharate begins to separate. When the molecular proportion of strontium to sucrose exceeds 3 : 1 the separation of sucrose as strontium bisaccharate is almost quantitative after 8 to 10 minutes' boiling. Strontium bisaccharate consists of white granular crystals of the formula $C_{12}H_{22}O_{11} \cdot 2\ SrO$. The compound is soluble in about 84 parts of boiling water but is insoluble in alcohol and in strongly alkaline aqueous solutions. For the complete precipitation of sucrose as bisaccharate the third molecule of strontium hydroxide can, therefore, be replaced by other alkalies such as sodium or potassium hydroxide.

When strontium bisaccharate is mixed with cold water it is decomposed, there being obtained a solution of the monosaccharate and free strontium hydroxide, the latter separating out in the crystalline form.

$$C_{12}H_{22}O_{11} \cdot 2\ SrO + H_2O = C_{12}H_{22}O_{11} \cdot SrO + Sr(OH)_2.$$

If the filtrate from the strontium hydroxide be saturated with carbon dioxide the monosaccharate is decomposed into sucrose and strontium carbonate. By evaporating the clear filtered solution, the sucrose is recovered in the crystalline form.

The method of precipitating sucrose as strontium bisaccharate is employed analytically for detecting sucrose in plant materials (p. 647); it also constitutes the basis of the strontium process for recovering sucrose from beet molasses. In the Scheibler† strontium process the diluted molasses and strontium hydroxide ($2\frac{1}{2}$ to 3 molecules of strontium to 1 of sucrose) are heated to 100° C. with constant agitation for about 30 minutes. The precipitated bisaccharate is then filtered off and washed hot with 10 per cent strontium hydroxide solution, until the

* Z. Ver. Deut. Zuckerind., **31,** 867.

† Ware's " Beet Sugar Manufacture and Refining " (1907), Vol. II, 502.

soluble impurities are removed and the precipitate is nearly white. The washed bisaccharate is then cooled for 1 to 2 days at a temperature of 5° to 10° C., when it decomposes, according to the preceding equation, into a solution of the monosaccharate and crystallized strontium hydroxide. The latter is separated by centrifugals and the solution of monosaccharate carbonated. The filtrate from the strontium carbonate (which is reconverted into strontium hydroxide) is a sucrose solution of about 97 per cent purity, and can be worked up directly into white sugar. The strontium bisaccharate process at the present time is largely replaced by the Steffens calcium trisaccharate method.

*Barium monosaccharate* is obtained by warming 100 parts of a 6 per cent aqueous sucrose solution with 20 parts of a 20 per cent barium hydroxide solution and allowing to cool unexposed to the carbon dioxide of the air. The compound may be prepared more easily by employing alcoholic instead of aqueous solutions of sucrose. Barium monosaccharate is a white crystalline compound with a composition corresponding to the formula $C_{12}H_{22}O_{11} \cdot BaO$. It is soluble in 47.6 parts of water at 15° C., easily soluble in aqueous sucrose solutions, but insoluble in alcohol or in aqueous barium hydroxide solutions. The compound is decomposed in contact with water by carbon dioxide, but the last traces of barium are precipitated only with difficulty; to facilitate the separation, the solution after carbonating may be heated with gypsum or ammonium sulphate, the traces of barium remaining in solution being precipitated as sulphate.

On account of the poisonous character of some of its salts, the use of barium for separating sucrose from molasses is forbidden in many countries. In Italy,* however, the barium saccharate method has proved successful and is still employed on a large scale, no injurious effects seeming to attend the use of the sugar thus prepared. In the Italian process the barium hydroxide solution is made up at 38 to 40 degrees Bé., and the molasses of 38 to 42 degrees Bé. added at a temperature of 45° to 50° C. The mixture is rapidly stirred and the barium monosaccharate, which soon becomes granular, allowed to settle. With normal molasses the barium hydroxide is used in the proportion of 1 molecule for each molecule of sucrose, plus an extra $\frac{1}{10}$ molecule for the non-sugars. The monosaccharate is then washed, decomposed in aqueous suspension with carbon dioxide and the filtrate from the barium carbonate evaporated to crystallization. The yield of sugar by the process is about one-third the weight of beet molasses.

In both the barium and strontium saccharate processes the barium

* Viewegh, Z. Zuckerind., Böhmen, **34**, 38.

and strontium are recovered and worked up again into hydroxides for continued use.

*Miscellaneous Metallic Compounds of Sucrose.* — In addition to the saccharates of the alkalies and alkaline earths a large number of compounds of sucrose with other metals have been prepared, such as saccharates of iron, aluminum, chromium, manganese, nickel, copper, lead and mercury. Some of the saccharates mentioned, as those of iron, are used medicinally. Lead saccharates of the formulæ $C_{12}H_{22}O_{11} \cdot PbO$, $C_{12}H_{22}O_{11} \cdot 2\ PbO$ and $C_{12}H_{22}O_{11} \cdot 3\ PbO$ are described in the literature, and these compounds are sometimes formed in the clarification of alkaline sucrose solutions by lead subacetate with introduction of considerable errors in the work of analysis. Soluble lead saccharates may affect the polarimetric reading, and precipitation of insoluble lead saccharates introduces a loss in the determination of sucrose.

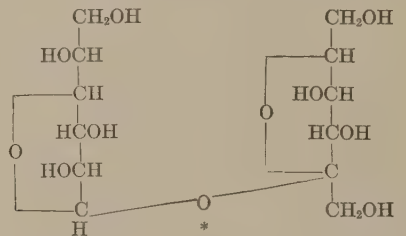
In connection with the formation of soluble saccharates there should be mentioned the property which sucrose has of preventing or retarding the precipitations of iron, aluminum, cobalt, nickel, copper and other metals from solution by means of sodium, potassium and ammonium hydroxides. In such cases metallic-sucrose complexes are formed, the exact constitution of which is not understood. The following are examples of the formulæ which have been given to a few such compounds as have been isolated, $C_{12}H_{22}O_{11} \cdot 5\ CuO + Na_2O$; $2\ C_{12}H_{22}O_{11} \cdot Fe_2O_3 + 2\ Na_2O$. Kahlenberg* from a study of the electric conductivity of solutions of such complexes believes that the metals do not exist in the dissociated condition of an ordinary salt solution but in the form of complex sucrose-metal ions.

**Tests for Sucrose.** — Characteristic qualitative tests for detecting small amounts of sucrose in presence of other sugars are wanting. In such cases the only certain means of identification is to precipitate the sucrose as one of its saccharates, preferably strontium bisaccharate, and to determine the optical and chemical properties of the sugar after liberation from its compound by means of carbon dioxide. The determination of specific rotation or reducing power before and after inversion with hydrochloric acid or invertase is also valuable as a means of identification. Sucrose in presence of inverting agents will of course give any of the reactions described for d-glucose and d-fructose. The deep violet coloration which even very dilute sucrose solutions give with $\alpha$-naphthol and sulphuric acid is also given by solutions of invert sugar. The violet coloration obtained by heating sucrose with an alkaline solution of cobalt nitrate was formerly regarded as a characteristic

* Z. physik. Chem., **17**, 616.

reaction; other sugars, however, give similar colorations so that the test is not reliable. The colorations which sucrose gives with morphine, codeine, aconitine, veratrine and other alkaloids in presence of sulphuric acid is also given by invert sugar; the same is also true of the blue coloration obtained by treating a sucrose solution with ammonium molybdate in presence of sulphuric acid.

**Configuration of Sucrose.** — A number of constitutional formulæ have been assigned to sucrose. The following arrangement by Wohl* and Fischer† is the one most generally preferred, although the exact configuration is still a matter of doubt:




The above arrangement contains no free aldehyde or ketone group which explains the non-reducing property of sucrose. The cleavage into d-glucose and d-fructose by inversion is supposed to take place at the O atom marked with a *.

The synthesis of sucrose from glucose and fructose has not as yet been accomplished.

**MALTOSE.** — Maltobiose. Malt sugar. Cerealose.

$C_{12}H_{22}O_{11}$.

The formation of a hitherto unknown sugar by the action of malt extract upon starch was noted by De Saussure‡ in 1819; some years later Dubrunfaut§ made a further study of the sugar and gave it the name maltose.

**Occurrence.** — Maltose is one of the most widely distributed disaccharides. The fact, however, that maltose is found in plants almost entirely as a transition, and not as a reserve, carbohydrate renders it difficult to isolate the sugar from ordinary plant substances in large amounts. In the vegetable kingdom maltose has been observed in the leaves of many plants, in young twigs and buds, in yeast, soja

* Ber., **23**, 2084.
† Ber., **26**, 2405.
‡ Ann. chim. phys. [2], **11**, 379.
§ Ann. chim. phys. [3], **21**, 178.

beans, rice and other substances; it is found most abundantly in starchy seeds at the time of germination when it is formed together with dextrin by the action of diastatic enzymes upon starch. The maltose, which is thus formed, is itself quickly hydrolyzed by other enzymes (*glucases*), so that the amount of free maltose occurring at any one time is relatively small. In the animal kingdom maltose has been observed in abnormal urines, in the intestinal tract, in the blood, liver and muscular tissues. Its occurrence in the animal organism is no doubt largely due to the action of amylolytic enzymes upon the starchy matter of the food.

**Diastatic Enzymes.** — Diastatic enzymes or amylases are widely distributed in both the vegetable and animal kingdoms. Aqueous extracts of barley, oats, rye, rice and other cereal grains as well as of many seeds; extracts of the blossoms, buds, leaves, roots, etc., of many plants, and also of many moulds, bacteria, fungi, lichens, etc., possess the property of converting starch into maltose and dextrin. In the animal kingdom amylases are found in the saliva (ptyalin), in the pancreatic juice (pancreatin), in the mucous secretions of the stomach and intestines, and in the liver, kidneys and other organs; their presence has also been reported in blood serum, in the lymph and even in urine and milk.

The fresh aqueous extract of many plant substances, such as starchy grains and seeds, have relatively but little diastatic power; if such grains and seeds, however, are moistened and allowed to germinate before making the extract, the starch converting power is found to undergo a marked increase. In such cases the amylase is supposed to be derived from an anterior substance, or *zymogen*, which is itself inactive. The following experiments by Salamon* show the increase in diastatic power during the germination of barley. The values are expressed in terms of Lintner's scale (p. 513) and are calculated in each case to a common basis of 2 per cent moisture.

| Day. | Diastatic power. | Day. | Diastatic power. |
|---|---|---|---|
| 1st | 6.5 | 8th | 90.4 |
| 2nd | 7.0 | 9th | 81.3 |
| 3rd | 10.7 | 10th | 77.4 |
| 4th | 49.2 | 11th | 85.5 |
| 5th | 78.1 | 12th | 108.2 |
| 6th | 74.1 | 13th | 125.0 |

* J. Fed. Inst. Brewing (1902), **8**, 2.

The results show a 20-fold increase in diastatic power during the 13 days of germination, although at certain stages there was an apparent decrease upon succeeding days.

**Malt.** — The diastases of germinated barley (malt) are of great importance in the brewing industry and have for this reason been studied more than any of the amylases. In the preparation of malt, raw barley is first steeped for 2 to 3 days in water at 10° to 13° C. until it has absorbed about 50 per cent its weight of moisture. The barley is then allowed to germinate for 9 to 12 days upon a floor in heaps about 1 foot in depth. The heaps are turned several times each day with wooden shovels in order to secure proper aëration and even distribution of temperature, the latter being maintained as nearly as possible at 15° C.; the grain is also sprinkled with water at intervals in order to maintain proper conditions of moisture. After germination has proceeded to the desired extent, as determined by the growth of the rootlets and acrospire, the fresh malt is transferred to a drying kiln, where it is heated at about 25° to 35° C. for the first day, at 40° to 45° C. for the second day, at 50° to 55° for the third day and at 60° to 65° for the fourth day. The kiln is then gradually raised to a final temperature varying from 85° to 110° C., according to the character of the malt desired. The gradual elevation of temperature is necessary, as diastase, like invertase and other enzymes, is extremely sensitive to heat in presence of moisture, although when perfectly dry the enzyme can withstand a much higher temperature. The diastatic power of the green malt is considerably reduced by the drying process, however, being only one-sixth to one-third of its original amount.

In the process of malting a series of important changes take place in the carbohydrates of the grain. In the first place a considerable amount of the conversion products of the starch are consumed by respiration, over 10 per cent of carbon dioxide being given off by the malt during germination. The maltose, which is produced by the action of the amylase upon the starch, is hydrolyzed into glucose by the glucase. Synthetic processes also take place; the reducing sugars absorbed by the aleurone cells and scutellum are built up into sucrose, the latter, in turn, as it contributes to the growth of the plant embryo, being hydrolyzed into glucose and fructose. The following analyses by O'Sullivan* give the per cent of different sugars in two samples of barley before and after germination.

* J. Chem. Soc. (1886), p. 58.

| | Barley No. I. | | Barley No. II. | |
|---|---|---|---|---|
| | Before germination. | After germination. | Before germination. | After germination. |
| | Per cent. | Per cent. | Per cent. | Per cent. |
| Sucrose | 0.9 | 4.5 | 1.39 | 4.50 |
| Maltose | ..... | 1.2 | ..... | 1.98 |
| Glucose | 1.1 | 3.1 | 0.62 | 1.57 |
| Fructose | ..... | 0.2 | ..... | 0.71 |
| | 2.0 | 9.0 | 2.01 | 8.76 |

**Preparation of Malt Diastase.** — For the preparation of diastase fresh green malt, or, when this is not available, fresh dry malt, is finely ground and digested for 2 to 3 hours with 5 parts of cold water. The filtered extract is then treated with a large excess of strong alcohol and the precipitated enzyme filtered off, redissolved in water and again precipitated with alcohol. The product thus prepared is washed with strong alcohol and ether, and then dried in vacuum over concentrated sulphuric acid. Diastase was prepared by Osborne* by precipitating the enzyme from malt extract by means of ammonium and magnesium sulphates, and then removing water-soluble impurities by dialysis. In this way the purity of the diastase was increased, but its activity was diminished owing no doubt to the removal of certain salts or other ingredients necessary for the activation of the enzyme.

**Properties of Malt Diastase.** — Diastase as ordinarily prepared consists of a white chalky powder, soluble in water to a clear frothy solution, but insoluble in alcohol and ether. It is precipitated from solution by tannic acid, magnesium sulphate and other salts. As prepared by Osborne diastase has the composition: C, 52.50 per cent; H, 6.72 per cent; N, 16.10 per cent; S, 1.90 per cent; O, 22.12 per cent; and ash, 0.66 per cent. Preparations of the enzyme give the ordinary tests for protein and also, according to Wroblewski,† for araban. Malt diastase has not been prepared, however, of sufficient purity to determine its exact composition.

## The Conversion of Starch

### conversion of starch by enzymes

In the study of the action of malt diastase upon starch, use has generally been made of malt extract rather than of the precipitated enzyme. Following the early work by Dubrunfaut, O'Sullivan,‡ in

* J. Am. Chem. Soc., **17,** 587. † Ber., **30,** 2289; **31,** 1127.
‡ J. Chem. Soc. (1872), **25,** 579.

1872, was the first to subject the action of malt diastase upon starch to a careful study, and since then a large number of investigators have made the question an object of research.

**Steps in Diastatic Conversion.** — Owing to the complexity of the starch molecule and the indefinite number of intermediate transition products which are formed between starch and maltose, such as amylodextrin, erythrodextrin, achroodextrin, maltodextrin, etc., the conversion of starch is a vastly more complicated reaction than the inversion of sucrose. It is generally agreed that malt diastase is a mixture of enzymes; the primary phase of starch conversion, consisting in the formation of soluble starch, is attributed to a liquefying enzyme or *cytase*; the remaining steps of the conversion are assigned to an *amylodextrinase*, which converts the soluble starch into dextrin, and to a *dextrinomaltase*, which converts the dextrin into maltose; an *amylomaltase* which converts soluble starch directly into maltose has also been supposed to exist. The difference in behavior of diastases from different sources is no doubt due in part to variations in amount of the constituent enzymes.

**Theory of Brown and his Coworkers.** — The conversion of starch into maltose by means of diastase under ordinary conditions is not complete, the reaction coming to a resting stage or condition of equilibrium. This is represented according to Brown and Heron,* and Brown and Morris† by the equation:

$$\underset{\text{Starch}}{10\ C_{12}H_{20}O_{10}} + 8\ H_2O = \underset{\text{Maltose}}{8\ C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{2\ C_{12}H_{20}O_{10}}.$$

Brown and Millar‡ in a later research show that the dextrin thus formed, upon prolonged treatment with diastase, breaks up into glucose as well as maltose, and to explain this and other facts give the equation:

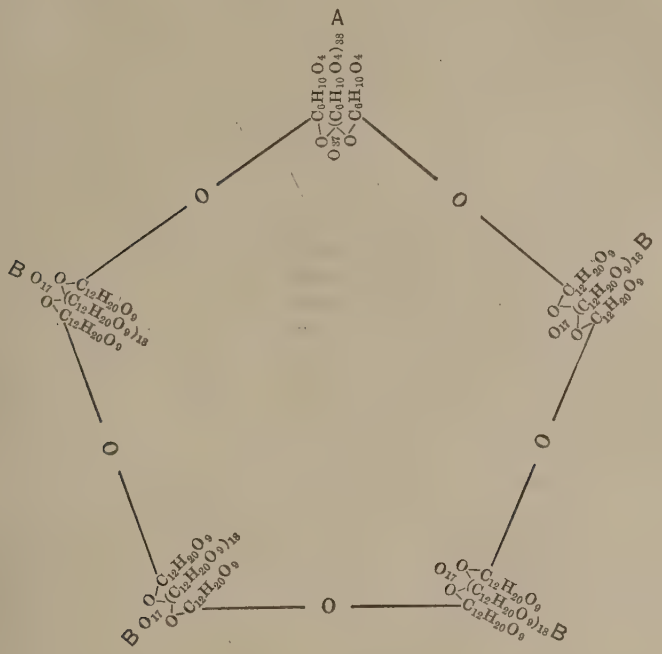
$$\underset{\text{Starch}}{100\ (C_{12}H_{20}O_{10})} + 81\ H_2O = \underset{\text{Maltose}}{80\ C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{(C_6H_{10}O_5)_{39} \cdot C_6H_{12}O_6}.$$

In other words 100 parts of starch yield 84.44 per cent of maltose. In this connection it is interesting to note that Sherman and Kendall§ found with pancreatin a tendency to equilibrium when the weight of maltose reached about 85 per cent of the initial weight of starch.

Starch according to Brown and Millar‖ has a molecular weight of

* J. Chem. Soc. Trans. (1879), **35**, 596.
† J. Chem. Soc. Trans. (1885), **47**, 527.
‡ J. Chem. Soc. Trans. (1899), **75**, 333.
§ J. Am. Chem. Soc., **32**, 1087.
‖ J. Chem. Soc. Trans. (1899), **75**, 333.

34,200 and consists of four similar maltan groups, $(C_{12}H_{20}O_{10})_{20}$, and one dextran group, $(C_6H_{10}O_5)_{40}$, combined in the following arrangement:




Upon hydrolysis with diastase the dextran complex *A* is split off, forming the stable dextrin 39 $(C_6H_{10}O_5) \cdot C_6H_{12}O_6$, which undergoes no further change under the ordinary conditions of conversion. The maltan complexes, B, on the other hand, are decomposed at the O linkages which join the $C_{12}$-groups and give rise, as the hydrolysis proceeds, to a series of maltodextrins of diminishing molecular weight with maltose as the final end product.

The above formula for starch and the theory of its conversion by diastase require, however, much additional confirmation before final acceptance. The example serves, however, as a good illustration of the complex problems which are involved.

**Theory of Maquenne and Roux.** — According to the recent conclusions of Maquenne and Roux* starch is to be regarded not as a com-

* Ann. chim. phys., **9**, 179.

pound but as a mixture of amylocellulose, or amylose, and amylopectin. The following conclusions are taken from the work of these authors:

*Amylocellulose* is identical with the granulose or soluble *amylose* of previous writers, and constitutes 80 per cent to 85 per cent of natural starch grains. Under amyloses are comprised those substances which are colored blue by iodine, are perfectly soluble in potash solution or superheated water and are saccharified without producing residual dextrins. The less condensed amyloses form the different soluble starches; the more highly condensed members are not soluble in the pure state except under pressure, at 150° to 155° C., but they form with the lower members eutectic mixtures, perfectly soluble in boiling water. The transformation of a lower amylose into a higher, less soluble homologue does not appear to take place outside the living cell. Amylose can assume at the same temperature two distinct forms; a soluble form immediately saccharified and colored by iodine, and a solid form which resists malt and gives no reaction with iodine. The latter is perhaps a polymeric form of the first. Solutions of amylose give with iodine a coloration about one-fourth more intense than those of natural starch. Starch grains are colored with iodine because a part of its amylose exists as a solid solution. Starch paste may retrograde, owing to the crystallization of amylose which the fresh paste holds in solution. By means of this property the crude amylose may be purified, and obtained in grains which resemble the original starch in microscopic appearance. Besides amylose, natural starch contains 15 to 20 per cent of a mucilaginous substance, *amylopectin*, which differs from amylose by swelling up without dissolving in boiling water or alkaline solutions, by being only very slowly saccharified by ordinary diastase and by giving no reaction with iodine. Starch paste is simply a perfect solution of amylose, rendered viscous by amylopectin. The saccharification of starch paste proceeds in two successive phases, a rapid phase of a few hours and a slower phase which lasts several days. The saccharification of the amylose makes up the rapid phase. The so-called residual dextrins, which accompany maltose in those worts which are imperfectly saccharified, result from the liquefaction and incomplete hydrolysis of the amylopectin. Malt extract is susceptible to auto-excitation as a probable result of the proteolysis of its albuminoids; this excitation is observed at all temperatures at which the amylase may exist undestroyed, and is always accompanied by a partial coagulation. Acids stimulate the activity of malt by producing the same condition of equilibrium which results from auto-excitation. Their effect, however, is generally less favorable than that of the latter, since the stability of the amylase

is diminished. Diastatic saccharification, to obtain a maximum effect, should be carried out in an alkaline medium. The optimum is obtained by first neutralizing the paste and then adding to the malt solution enough sulphuric acid to neutralize from one-third to one-half the alkalinity present, using methyl orange as indicator. The second or slow phase of ordinary saccharification corresponds to the hydrolysis of the residual dextrins (amylopectin) by means of a special diastase (dextrinase) formed during the auto-excitation of the malt.

The following results by Maquenne and Roux show the action of malt extract at 50° C. upon starch paste and upon a solution of amylose:

| Time. | Percentage of maltose on original starch substance. | | Time. | Percentage of maltose on original starch substance. | |
|---|---|---|---|---|---|
| | Starch paste. | Amylose. | | Starch paste. | Amylose. |
| | Per cent. | Per cent. | | Per cent. | Per cent. |
| 5 minutes | 66.7 | 94.4 | 1.5 hours | ..... | 100.0 |
| 15 minutes | 74.9 | 98.1 | 2 hours | 81.1 | 100.1 |
| 30 minutes | 76.9 | 99.7 | 2.5 hours | ..... | 100.0 |
| 45 minutes | ..... | 99.6 | 3 hours | 82.0 | 101.4 |
| 1 hour | 79.0 | 99.7 | 28 hours | 91.8 | 104.2 |

It is seen that the yield of maltose from amylose is almost the theoretical (105.5 per cent). The apparent halt in the reaction with starch paste, when about 80 per cent maltose is formed, is the same as that indicated by Brown and Morris, and is explained by Maquenne and Roux on the assumption that the saccharification of the amylose is then nearly complete.

The numerous other hypotheses which have been proposed to explain the saccharification of starch with malt extract show the same divergence of opinion as exists between the views of Brown and Millar, and of Maquenne and Roux. The only points of general agreement are that the principal products of conversion by diastase are maltose and dextrin, and that this residual dextrin by a process of slow hydrolysis is also eventually converted into maltose.

While starch, under special methods of preparation, suitable conditions of temperature, proper activation of diastase and sufficient interval of time, may undergo an apparent complete conversion into maltose, the question is still open whether the final product of such conversion is pure maltose or a mixture, consisting largely of maltose with a certain amount of isomaltose, glucose and dextrin, whose combined rotations and reducing powers agree closely with those of maltose. It is not surprising, therefore, when the mixed character of the enzymes

in malt diastase and the complexity of the various reactions are considered, that the efforts to establish a simple law of mass action for starch conversion, such as that observed for the inversion of sucrose, should have met with failure. The fact that the starches of different vegetable origin have in all probability a different molecular structure still further complicates the problem.

**Influence of Temperature upon Diastatic Conversion.** — The optimum temperature for saccharification of starch by malt diastase is about 45° C., although the point of maximum conversion may lie considerably above or below 45° C., according to conditions. Diastase solutions undergo a great reduction in activity upon warming above 60° to 65° C.; above 75° C. the saccharifying power is completely destroyed.

*Effect of Mashing at High and Low Temperature.* — The effect of temperature upon the different enzymes of malt extract is variable. Malt extract, which has been heated to 75° C. and which has thus lost its saccharifying power, still liquefies starch as strongly as ever, converting it almost quantitatively into dextrin. The optimum temperature for the liquefying and dextrin-forming enzymes of malt extract lies, in fact, between 70° and 75° C. It is evident, therefore, that the yield of maltose and dextrin from starch can be controlled to a considerable extent by the temperature of conversion, and this fact is utilized in the technical operations of brewing. Mashing at 70° C. will produce more dextrin, and hence give a beverage of greater body (solid content), than mashing at 45° C. Mashing at 45° C., on the other hand, will produce more maltose and hence give a beverage of higher alcohol content than mashing at 70° C. The composition of worts by the high and low temperature methods of mashing is given in the following table:*

| Character of Wort. | Wort No. 1 (low temperature). 1 hour at 45° C. 20 min. at 45°–80° C. 25 min. at 80° C. | | Wort No. 2 (high temperature). 10 min. at 60°–80° C. 25 min. at 80° C. | |
|---|---|---|---|---|
| | Maltose. | Dextrin. | Maltose. | Dextrin. |
| Grams in 100 c.c. of wort.... | 8.93 | 2.17 | 7.25 | 3.14 |
| Per cent in dry extract..... | 70.39 | 17.00 | 58.34 | 25.30 |

*Restriction of Malt Extract.* — Ling and Davis † found that when malt extract is heated above 55° C. not only does the saccharifying

* F. Fischer's "Handbuch der chem. Technologie" (1902), II, 337-8.

† J. Fed. Inst. Brewing, **8**, 475 (1902).

power undergo a decrease but glucose begins to be formed as one of the products of conversion. Malt extracts whose saccharifying power has been weakened by heating are said to be restricted; the maximum yield of glucose (12 per cent of total conversion products) is obtained by malt extracts which have been heated at 68° to 70° C. for 15 to 30 minutes. Ling and Davis explain the phenomena of restriction by assuming that an alteration has been produced in the enzyme molecule so that glucose becomes the end product of conversion instead of maltose. Prior,* however, explains the facts by assuming that a glucose-forming enzyme (amyloglucase) exists in malt extract and is more resistant to heat than the amylomaltase.

The presence of glucose in malt sirups was at one time regarded as an evidence of adulteration with commercial dextrose or glucose sirup. Perfectly pure malt sirups† may contain, however, several per cent of glucose if the diastase of the malt has undergone restriction. A large amount of glucose may also be derived from the malt itself, as shown by the analysis of cold water extracts (see table, page 511).

**Influence of Acids, Alkalies, Salts, Etc., Upon Amylolytic Action.**— The addition of acids in minute amounts accelerates the activity of malt diastase; in larger amounts acids have a marked retarding influence upon the enzyme, the degree of retardation following apparently the same rule noted for invertase and being proportional to the concentration of hydrogen ions.

Alkalies and alkaline-reacting salts are very injurious to the action of malt diastase if present beyond the merest trace. A perfectly neutral medium is believed by some to be the most favorable for diastatic action, while others maintain that the reaction should be slightly acid or even faintly alkaline. The explanation of these differences of opinion is probably the same as that given by Sherman and Kendall for pancreatin (p. 694).

Small amounts of the neutral salts of the alkalies and alkaline earths (chlorides, sulphates, phosphates, etc.), usually accelerate the activity of malt diastase, frequently to a very marked degree. Calcium and barium chlorides seem, however, to have a retarding influence. Addition of sulphates or of salts of the heavy metals in large amounts check the activity of the enzyme, owing probably to precipitation. Traces of silver nitrate or of mercuric chloride destroy diastatic action completely.

Of organic substances albumin and asparagine seem to favor diastatic action. Alcohol in slight amounts exerts no appreciable influence;

* Wochenschr. f. Brauerei, **21**, 349 (1904).

† Long and Rendle, Analyst (1904).

in larger quantities, however, the activity of the enzyme is reduced, owing to destruction or precipitation. Formaldehyde in amounts exceeding 0.005 per cent has a marked retarding influence.

The destructive action of heat, acids, alkalies, salts, alcohol, etc., upon diastase is considerably reduced, if starch or its conversion products, maltose and dextrin, are present; the protective action of these substances is similar to that noted for sucrose and fructose upon invertase (p. 675).

**Action of Other Amylases.** — As to the action upon starch of other diastases than those of malt, mention will be made only of takadiastase and of the animal amylases ptyalin and pancreatin.

*Takadiastase*, the best known example of a fungus diastase, has been employed in Japan for an unknown period of time in saccharifying starchy materials for the production of alcoholic beverages. The enzyme has been separated by Takamine* and is now a standard pharmaceutical preparation for the relief of starch indigestion. The patented process of Takamine for its production is as follows:

Wheat bran is steamed and then, after cooling, sown with the spores of the mould *Aspergillus oryzæ*. The moist bran is kept at a temperature of about 25° C.; in about 24 hours the spores have germinated and the growth of mycelium becomes visible; after about 48 hours, when the production of diastase has reached its maximum, further growth of the mould is checked by cooling. The material in this condition, consisting of bran felted together by the threads of mycelium, is called "taka-koji" in Japan, where it is used in the same manner as malt. To prepare the enzyme "taka-koji" is extracted with water, the aqueous extract concentrated at low temperature, and then treated with an excess of alcohol. The takadiastase, which is precipitated, is filtered off, pressed and carefully dried; the enzyme as thus prepared consists of a white powder, easily soluble in water, and has a very strong converting power.

Stone and Wright† have made a comparative study of the action of a pharmaceutical preparation of takadiastase at 40° C. and of a laboratory preparation of malt diastase at 60° C. Following the conversion of potato starch it was noted that the takadiastase was more rapid in its action during the initial conversion than malt diastase, there being an almost immediate change from the typical blue of the starch-iodine compound to the reddish and violet tints. "On the other hand the complete conversion of the starch into forms which no

* Am. Jour. Pharm., **70**, No. 3; J. Soc. Chem. Ind., **17**, No. 2.
† J. Am. Chem. Soc., **20**, 639.

longer gave color reactions with iodine was effected much earlier by the malt diastase." The same results were obtained when the saccharification was followed by studying the decrease in specific rotation and the increase in copper reducing power.

The results of the work of Stone and Wright show that for a given short period (15 minutes to 2 hours) the saccharifying power of the takadiastase was superior to that of the malt-diastase preparation, but that for the complete saccharification of starch, especially in cellular materials, where the starch granules were retained and not readily brought into solution, the malt diastase was more effective; the cellular residues after 7 hours' digestion with takadiastase at 40° C. still gave the iodine reaction when observed under the microscope, while the residues after 7 hours' digestion with malt diastase at 60° C. gave no such reaction. These results were obtained, however, with only one set of enzyme preparations and under only one set of conditions. With different enzyme preparations, and other conditions of temperature, activation, etc., than were employed by Stone and Wright, different results would no doubt be obtained.

*Ptyalin*, the amylase of saliva, plays an important part in the digestion of starchy foods; it occurs most abundantly in the saliva of herbivorous animals. Ptyalin can be prepared from saliva by precipitating with alcohol, as described under invertase and diastase. The optimum temperature for its action is about 40° C., at which point starch paste is saccharified almost immediately. Raw starch in the process of mastication is also quickly converted into 80 to 100 per cent sugar.* Ptyalin, similar to diastase, contains several enzymes, a liquefying enzyme, an amylomaltase, an amyloglucase, etc. In some cases the product of conversion seems to be almost pure maltose; in other cases a mixture of maltose, glucose and isomaltose (?). The variability of its action is no doubt due to differences in the amount of the constituent enzymes. Minimal quantities of acid (under 0.002 normal) accelerate the action of ptyalin; large amounts of acid have a retarding influence. Alkalies and alkaline reacting salts are depressing in their action. The chlorides, sulphates, etc., of the alkalies also retard the activity of ptyalin if present in large amounts.

*Pancreatin*, the amylase of the pancreatic juice, has recently been subjected to a careful study by Sherman† and his coworkers and the following facts are cited from their work.

Commercial pancreatin, which had been freed from accompanying

* Müller, Chem. Centralbl. (1901), 637.

† J. Am. Chem. Soc., **32**, 1073, 1087; **33**, 1195.

salts by dialysis, was without action upon dialyzed soluble starch. When, however, a neutral salt was added the enzyme was activated as shown in the following experiment:

0.35 mg. pancreatin in 50 c.c. of 1 per cent dialyzed starch at 40° C. for 1 hour showed for various additions of salt the following activities, expressed by weights of reduced cuprous oxide obtained upon heating with Fehling's solution:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sodium chloride, mgs. | 0.01 | 0.1 | 1.0 | 10 | 30 | 60 | 90 | 121 |
| Cuprous oxide, mgs. | 0 | 10 | 51 | 87 | 91 | 86 | 85 | 85 |

Experiments with potassium and ammonium chlorides gave similar results. The presence of salts are, therefore, not only helpful but are essential to the action of the enzyme.

Excess of acid or alkali destroys the activity of pancreatin. The influence of acid and alkalies in minimal amounts is given in the following table which shows the action of 0.125 mg. pancreatin upon 0.25 gm. soluble starch at 40° C., sufficient NaCl being added to activate the enzyme. Results are given as milligrams of reduced cuprous oxide.

TABLE C

*Conversion of Starch by Pancreatin*

*(Effect of Added Acid and Alkali on Solutions Containing Neutral Electrolyte)*

| Added acid or alkali. | Time. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 min. | 30 min. | 1 hr. | 2 hrs. | 3 hrs. | 5 hrs. | 25 hrs. |
| 8 c.c. | | | 0 | | | | |
| 6 c.c. | | | 0 | | | | |
| 4 c.c. 0.01 normal sulphuric acid | | | 3 | | 7 | | |
| 3 c.c. per 100 c.c. | | | 87 | 151 | | 222 | 277 |
| 2 c.c. | | | 153 | 218 | | 251 | 272 |
| 1 c.c. | | | 223 | 242 | | 254 | 271 |
| Neutral | | | 227 | 243 | | 254 | 270 |
| 1 c.c. | 143 | 207 | 235 | | 240 | 252 | |
| 2 c.c. | 156 | 204 | 226 | | 244 | 250 | |
| 3 c.c. | 124 | 191 | 214 | | 241 | 244 | |
| 4 c.c. | | | 200 | 222 | | 239 | 256 |
| 6 c.c. 0.01 normal sodium hy- | | | 154 | 196 | | 232 | 250 |
| 8 c.c. droxide per 100 c.c. | | | 124 | 186 | | 227 | 250 |
| 20 c.c. | | | 19 | | 40 | 163 | |
| 30 c.c. | | | 11 | | 18 | 40 | |
| 40 c.c. | | | 3 | | 9 | 11 | |
| 50 c.c. | | | 0 | | 4 | 6 | |

It is seen that the highest degree of saccharification is obtained in faintly acid solution at the end of 25 hours; on the other hand the conversion during the first hour is more rapid in faintly alkaline solution. The influence of alkalies seems to depend upon the initial concentra-

tion of starch, the effect at first being to accelerate, and then, as the starch is changed, to retard the speed of saccharification. For a short period of time an alkaline (and for a long period of time an acid) reaction gives the maximum yield of maltose. This is no doubt one explanation for the variable conditions reported by different investigators for the optimum conversion of starch by pancreatin and other amylases.

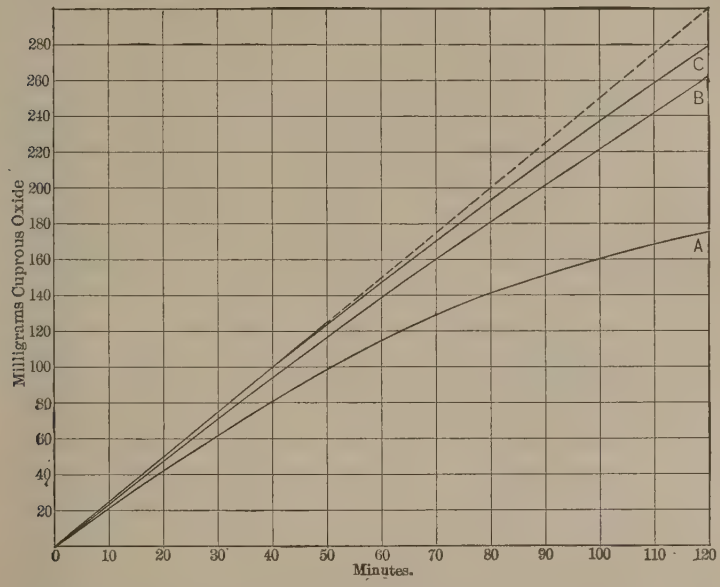



Fig. 199. — Time curves showing effect of concentration of soluble starch upon the rate of conversion by pancreatin. *A*, curve for 0.5 per cent; *B*, curve for 2.0 per cent; and *C*, curve for 4.0 per cent starch solution. (Sherman and Kendall.)

The effect of concentration of starch upon the rate of conversion by pancreatin is shown in Fig. 199. A constant quantity of enzyme was allowed to act upon starch solutions of 0.5 per cent, 2.0 per cent and 4.0 per cent strength.

It is seen that the initial speed of conversion for a constant amount of enzyme is the same for the different concentrations, but that this speed diminishes more rapidly the smaller the initial concentration of starch. With increasing concentration of starch the time curves approach a straight line.

The effect of temperature upon the activity of pancreatin is shown

in the following table: 0.5 mg. of enzyme was allowed to act upon 100 c.c. of starch solution for 1 hour, in presence of a sufficient amount of activating salts.

| Temperature. | Cuprous oxide. | Temperature. | Cuprous oxide. |
|---|---|---|---|
| Deg. C. | Mgs. | Deg. C. | Mgs. |
| 21 | 65 | 50 | 345 |
| 30 | 122 | 55 | 378 |
| 40 | 238 | 60 | 256 |
| 45 | 298 | 65 | 66 |

" Between 20° C. and 40° C. the speed is about doubled every 10° C., in accordance with van't Hoff's rule for normal chemical reactions; between 40° C. and 55° C. the acceleration is less, but temperature still has a great effect. Beyond 55° C., where the maximum activity was obtained, the rate of change decreases very rapidly." When no activating salts are present increase of temperature above 20° C. depresses the activity of pancreatin and this " may be due to the fact that water itself has a greater paralyzing effect at the higher temperature." " Pure water, acting on pancreatic amylase free from neutral electrolyte, gradually destroys it, but if a trace of salt and alkali are present it will remain active for a long time."

The saccharification of starch with pancreatin is not usually complete. Sherman and Kendall* found that " working with 1 per cent starch, however favorable the conditions of salt and alkalinity, and however large the amount of enzyme, the hydrolysis tended to come to equilibrium when the weight of maltose reached about 85 per cent of the initial weight of starch."

**Converting Power of Amylases of High Activity.** — Sherman and Schlesinger† found by extracting dry commercial pancreatin with 50 per cent alcohol, precipitating the amylase with absolute alcohol or alcohol-ether, redissolving in 50 per cent alcohol, dialyzing against 50 per cent alcohol in presence of maltose (to protect the enzyme against deterioration) and then reprecipitating, that a very pure amylase resulted which had a diastatic power at 40° C. of 3480 on Sherman's scale, corresponding to over 5000 on Lintner's scale or to $D_{30'}^{280°} = 500{,}000$ on Wohlgemuth's scale. This preparation acting at 40° C. on soluble starch formed 6000 times its weight of maltose in 20 minutes and 211,000 times its weight in 30 hours. It digested 400,000 times its weight of starch to the " erythrodextrin stage " in less than 22 hours, and to products giving no reaction with iodine in 48 hours."

* J. Am. Chem. Soc., **32**, 1087. † J. Am. Chem. Soc., **33**, 1195.

### CONVERSION OF STARCH BY ACIDS

When starch is heated with acids it is converted into glucose according to the equation:

$$\underset{\text{Starch}}{(C_6H_{10}O_5)n} + nH_2O = \underset{\text{d-Glucose.}}{nC_6H_{12}O_6}.$$

With strong acids the conversion may be made in dilute solution at 100° C.; with weak acids it is necessary to employ a higher concentration of acid and, in certain cases, to conduct the hydrolysis under pressure at temperatures considerably above 100° C. in order to secure complete conversion into glucose.

While the acid conversion of starch in its final phase proceeds very closely according to the above equation, the different stages of the conversion, as starch to dextrin, dextrin to maltose, maltose to glucose etc., present the same complexities and uncertainties observed in the conversion by diastase.

**Formation of Maltose During Acid Conversion.** — The best recognized products of the incomplete conversion of starch by acid are glucose and dextrin. The occurrence of maltose among the products of incomplete acid conversion has been a subject of much dispute; many chemists hold that, while maltose exists as an intermediate product in the conversion, it is hydrolyzed into glucose almost as quickly as formed, and that the apparent values found for the specific rotation and reducing power of maltose are in reality only the values for mixtures of glucose and dextrin. Maltose has been separated, however, as its osazone by Rolfe and Haddock* from the acid conversion products of starch and its presence has also been recognized by Sieben,† Vogel,‡ Weber and MacPherson§ and other investigators.

**Formation of Dextrins and Reversion Products.** — A great difference of opinion also exists as to the nature of the dextrins which are formed during the acid conversion of starch. Some chemists believe that only one dextrin (of about $[\alpha]_D + 200$) is formed; other chemists, however, hold that there exists a series of dextrins having different rotations and reducing powers and resembling the amylo-, erythro-, achroo- and maltodextrins of diastatic conversion. An additional complication is the formation of *reversion* products, — especially when the starch is hydrolyzed by more concentrated acid, — a part of the glucose being recombined to form isomaltose and different synthetic dextrins.

* J. Am. Chem. Soc., **25**, 1015.
† Z. Ver. Deut. Zuckerind, **34**, 837.
‡ Chem. Ztg., **19**, 408.
§ J. Am. Chem. Soc., **17**, 312.

**Manufacture of Commercial Glucose and Dextrose.** — The acid conversion of starch is of great technical importance, being used in the manufacture of starch sirups (commercial glucose) and commercial dextrose or grape sugar. In the manufacture of glucose sirups starch (usually corn starch) is mixed with water to a cream of about 20 degrees Bé. and then heated with about 0.06 per cent its weight of hydrochloric acid in copper converters under a pressure of about 30 pounds. The conversion is controlled by iodine tests and requires about 1 hour. The liquid, which has a density of about 18 degrees Bé., is then neutralized with sodium carbonate, filtered through bone black and evaporated in vacuum pans to the required density, which varies between 41 and 45 degrees Bé. according to the demands of the trade. In some factories sulphuric acid is used as the hydrolyzing agent, in which case calcium carbonate is used for neutralizing.

In the manufacture of dextrose or grape sugar, a much larger amount of acid is used for conversion, — frequently 1 per cent or more of the weight of starch, — and the heating is continued until all dextrin is hydrolyzed, the end point being indicated by the absence of a precipitate when a little of the solution is poured into strong alcohol. The liquid is then neutralized, filtered through bone black, evaporated in vacuum to a thick sirup and poured into pans or moulds where it is allowed to solidify; the contents of the pans are usually "seeded" or primed with a little crystallized dextrose to hasten the crystallization.

*Rolfe* Upon the Acid Conversion of Starch.* — The progression of the hydrolysis of starch by means of acid is described by Rolfe as follows:

"The gradual disintegration of the starch molecule and the different stages of the hydrolysis of the products of this disintegration all go on at the same time, so that the final products of hydrolysis are always present in very small quantity even at the initial stages of the hydrolysis. The progression of the hydrolysis manifests itself in the following characteristics: The starch paste gradually loses its colloidal nature and passes over to a thin sirup, its viscosity continually decreasing. The dissolved carbohydrate increases in weight but the density effect of a given weight of carbohydrate in a given volume of solution continually decreases. The specific rotation of the carbohydrate, taken as a whole, likewise decreases, while its cupric-reducing

* Rolfe, "The Polariscope" (1905), p. 175. See also the paper by Rolfe and Defren, "An Analytical Investigation of the Hydrolysis of Starch by Acids." J. Am. Chem. Soc. **18**, 869.

power increases, these values progressively approaching those for dextrose.

"The iodine tests are also characteristic; a few drops of iodine solution giving, with the hydrolyzed solutions, at ordinary temperature, colors which change progressively as the hydrolysis proceeds from the deep sapphire blue of the unchanged starch, first to violet and reddish purple, then to a rose madder, and then to a reddish brown, growing lighter as the conversion proceeds, till at a later stage, but before hydrolysis is complete, the iodine gives no color reaction."

**Preparation of Maltose.** — Maltose is best prepared by the following method of Herzfeld;* 500 gms. of starch are stirred into 500 c.c. of water at 30° C., 4 liters of boiling water are then added and the paste which is formed cooled to 60° C. Malt extract, prepared by digesting 100 gms. of finely ground malt with 500 c.c. of water at 30° to 40° C., is then added and the liquid kept at 60° C. for 2 hours. The solution is then filtered, evaporated to 750 c.c. and 87 per cent alcohol added until the alcoholic strength of the solution is between 60 and 70 per cent. After standing 24 hours in a closed vessel, the alcoholic solution is decanted from the precipitated dextrin; the alcohol is distilled and the solution evaporated to a thin sirup. The latter is then extracted with 1 liter of 87 to 90 per cent alcohol, by boiling with successive portions under a reflux condenser. The combined extracts, containing the maltose, are set aside in a closed flask for 24 hours, filtered from deposited impurities, evaporated to a sirup and then allowed to stand in an open dish at 20° to 25° C. After several weeks' standing the maltose will crystallize either in white concretions or as fine microscopic needles. If the sirup be spread in a thin layer, and then primed with a few crystals of maltose, and stirred at frequent intervals, crystallization will be complete in about 8 days. The crystalline mass is then rubbed to a paste with cold methyl alcohol, pressed between filter paper and recrystallized from hot methyl alcohol, using bone black.

**Properties of Maltose.** — Maltose as ordinarily prepared is obtained as the monohydrate $C_{12}H_{22}O_{11} + H_2O$, consisting of fine prismatic needles, which melt upon rapid heating at about 100° C. The water of crystallization is removed only with great difficulty. Upon heating in the air at 100° to 110° C., the water is slowly evolved, but with decomposition of the sugar. If the monohydrate is first dried over concentrated sulphuric acid and then slowly heated up to 90° C. over a strong dehydrating agent (as phosphorus pentoxide), under the vacuum of a mercury pump, the last traces of water are finally removed. The

* Neue Z. Rübenzuckerind, **3,** 150; Ann., **220,** 200.

anhydrous maltose, as thus prepared, consists of a white amorphous mass and is extremely hygroscopic, absorbing moisture upon exposure to the air with the same avidity as calcium chloride.

*Specific Rotation.* — The values given in the literature for $[\alpha]_D$ of maltose range from + 136 to + 150, the extreme figures being due no doubt to impure preparations of sugar contaminated with water of hydration or with higher rotating dextrins. The values for carefully crystallized and dehydrated preparations of maltose range from about + 137 to + 139, the variations in this instance being due to the influence of temperature and concentration. For ordinary purposes the mean value + 138 may be used. The general equation for concentration and temperature is given on page 181.

The specific rotation of maltose hydrate is 95 per cent of that for the anhydride.

Freshly prepared solutions of maltose exhibit mutarotation, the initial rotation, however, as Dubrunfaut first observed, being less than the constant value. Parcus and Tollens* found for 1.9074 gms. of maltose anhydride dissolved to 20 c.c. the following values:

| | |
|---|---|
| 8 minutes after solution | + 119.36 |
| 15 minutes after solution | + 121.01 |
| 30 minutes after solution | + 123.35 |
| 1 hour after solution | + 128.07 |
| 2 hours after solution | + 132.97 |
| 5 hours after solution | + 136.52 |
| 24 hours after solution | + 136.96 |

Schulze and Tollens† noted for 2 gms. of maltose hydrate dissolved to 20 c.c. an initial rotation of + 95.83 and a constant value of + 129.42. An addition of a trace of ammonia destroys the mutarotation and gives the constant value within a few minutes. As first shown by Brown and Morris‡ maltose at the moment of its formation from starch by means of diastase exists in the low rotating form.

**Reactions of Maltose.** — Maltose reduces Fehling's solution about 60 per cent as strongly as d-glucose. If after the end of the reduction the solution is acidified with hydrochloric acid and then again boiled with Fehling's solution, a second quantity of copper is reduced, in about half the original amount. Maltose is distinguished from the simple reducing sugars by its failure to reduce Barfoed's copper acetate solution (p. 336).

*Oxidation of Maltose.* — By the action of bromine in aqueous solution maltose is oxidized to maltobionic acid. This was obtained by Fischer and Meyer§ as a sirup, which upon boiling with 5 per cent sulphuric

* Ann., **257**, 173.
† Ann., **271**, 219.
‡ Chem. News, **71**, 123.
§ Ber., **22**, 1941.

acid was hydrolyzed into d-glucose and d-gluconic acid. This reaction and the reducing properties of maltose indicate that one of the glucose radicals of maltose has its aldehyde group in the free reactive condition. The oxidation to maltobionic acid is shown as follows:

$$\underbrace{C_5H_{10}O_5CH-O-C_5H_{10}O_4\cdot CHO}_{\text{Maltose}} + O = \underbrace{C_5H_{10}O_5CH-O-C_5H_{10}O_4\cdot COOH}_{\text{Maltobionic acid}}$$

(d-Glucose radical; d-Glucose radical; d-Glucose radical; d-Gluconic acid radical)

Oxidation of maltose with nitric acid gives d-saccharic acid.

*Action of Alkalies.* — Maltose upon heating with dilute alkalies undergoes an almost complete loss of optical activity, the sugar molecule undergoing partial hydrolysis and rearrangement* with formation of d-glucose, d-mannose and other products of unknown composition. Upon warming with concentrated alkalies maltose solutions turn dark brown, the maltose being broken up into lower decomposition products among which lactic acid is the most important. The lactic acid thus formed consists, according to Duclaux,† of a mixture of d- and d, l-lactic acid, and under favorable conditions may equal 50 per cent of the original weight of maltose.

*Action of Acids.* — Maltose on heating with 2 to 3 per cent hydrochloric or sulphuric acid for several hours upon a boiling water bath is hydrolyzed into d-glucose.

$$\underbrace{C_5H_{10}O_5CH-O-C_5H_{10}O_4CHO}_{\text{Maltose}} + H_2O = 2\ \underbrace{C_5H_{11}O_5CHO}_{\text{d-Glucose.}}$$

The hydrolysis proceeds much more slowly than the inversion of sucrose and the yield of d-glucose is nearly but not absolutely quantitative, being 98 per cent to 99 per cent of the theoretical; the 1 to 2 per cent loss is due to destruction of sugar with formation of levulinic acid, humus substances, reversion products, etc.

The hydrolysis of maltose by acids, according to Sigmond,‡ follows Wilhelmy's law for a reaction of the first order, the velocity constant $k$ increasing with concentration and rising temperature. W. A. Noyes§ and his coworkers found, however, that the values for $k$, as determined from copper reducing power, show a rapid decrease in the later stages of hydrolysis. The hydrolyzing power of the different acids upon maltose follows the same order observed by Ostwald for the inversion of sucrose.

**Fermentation of Maltose.** — In so far as the various yeasts, moulds and bacteria secrete the enzyme *maltase* or *maltoglucase* they

* Rec. trav. Pays-Bas, **14,** 156, 203.
† Chem. Centralbl. (1894), 169.
‡ Z. physik. Chem., **27,** 386.
§ J. Am. Chem. Soc., **26,** 266.

ferment maltose in the same manner as d-glucose. In case, however, the organism does not form maltoglucase, as, for example, *Saccharomyces Marxianus*, maltose is not fermented. *Maltoglucase* is formed in large amount by many varieties of yeasts, a classification of which is given in Table CII, page 714.

Ordinary beer yeast is especially rich in maltoglucase and ferments maltose with the same ease and rapidity as d-glucose, 100 parts of maltose anhydride, according to Jodlbauer, yielding 51.08 per cent alcohol, 49.04 per cent carbon dioxide, 3.95 per cent succinic acid and glycerol and 0.90 per cent of other products — a total of 105 per cent, which corresponds to the theoretical yield of d-glucose from maltose.

*Maltoglucase.* — The preparation of maltoglucase presents considerably more difficulty than that of invertase owing to the greater resistance of the enzyme towards extraction and its greater sensitiveness towards antiseptic agents. According to Fischer and Lindner* the enzyme is best prepared by washing the yeast with water, drying upon an unglazed earthen-ware plate for 3 days at ordinary temperature, then pulverizing the dried yeast in a porcelain mortar and extracting with 20 times its weight of water for 20 hours at 33° C. As antiseptic agents thymol or toluol† are less injurious than chloroform. Maltoglucase has not been isolated as yet in the pure form; its solutions and preparations are always contaminated by other enzymes, (invertase, amylase, etc.). The temperature optimum for the activity of maltoglucase, according to Lintner and Kröber,‡ is about 40° C.

In addition to yeast different varieties of Mucor, Aspergillus, Monilia, Torula, as well as various Amylomyces, form maltoglucase and ferment maltose with production of alcohol.

Maltoglucases are also found in many grains, in malt and in most starchy seeds during germination, in peas, beets, potatoes, in the green leaves of many plants and in other vegetable organs; the enzyme occurs mostly associated with amylases. The same association also exists in the animal kingdom, maltoglucases being found in saliva, in pancreatic juice and in the secretions of the intestines, liver, etc.

Maltose is fermented by nearly all the lactic and butyric acid organisms in the same manner as d-glucose. The same is also true of most oxidizing fermentations. *Citromyces Pfefferianus* yields about 50 per cent citric acid from maltose, *Bact. oxydans* produces acetic acid. Oxalic acid, butyl and other alcohols and ethyl acetate are among the products of special fermentations. *Leuconostoc mesenterioides* produces

* Ber., **28**, 984. † Fischer, Ber., **28**, 1429.
‡ Ber., **28**, 1050.

lactic acid from maltose but does not produce dextran as is the case with sucrose.

**Compounds of Maltose.** — Maltose contains a free aldehyde group and the sugar is consequently much more reactive than sucrose, forming methyl and ethyl maltosides, mercaptals, ureides, etc., in the same manner as the simple reducing sugars. In the same way maltose reacts with phenylhydrazine and its substituted derivatives forming a large number of hydrazones and osazones. The most important of the latter from the analytical standpoint is maltose-phenylosazone, $C_{12}H_{20}O_9(N \cdot NHC_6H_5)_2$, which is formed by heating maltose solutions with an excess of phenylhydrazine acetate for 1 hour. The osazone, owing to its solubility in hot water, does not crystallize out until after cooling, when it separates in the form of fine yellow needles; the compound after recrystallizing melts upon rapid heating at 206° C. with decomposition. Maltose-phenylosazone is only slightly soluble in cold water, is soluble in 75 parts hot water, in 150 parts hot absolute alcohol, but is insoluble in ether. It undergoes decomposition upon long heating with boiling water, so that the action of hot solutions must not be prolonged; if the heating is continued too far the melting point of the osazone may be reduced to 150° C. Maltosazones of low melting point are also obtained when the reaction is carried out with too little phenylhydrazine or in too small an amount of water. The melting point and character of the osazone are also greatly modified by other sugars and especially by the different dextrins of starch conversion.

Maltose forms with acetic anhydride a number of acetates of which the octacetate, $C_{12}H_{14}(C_2H_3O)_8O_{11}$, is the most characteristic; it consists of white crystals with bitter taste, melting at 157° to 159° C., and giving in benzol solution $[\alpha]_D = +76.54$, in chloroform $[\alpha]_D = +61.01$ and in alcohol $[\alpha]_D = +60.02$.

Maltose forms with alkalies and alkaline earths a series of maltosates, none of which, however, has the importance of the corresponding sucrose derivatives.

Upon treatment with hydrocyanic acid maltose forms a nitrile which yields after saponification maltose carboxylic acid; the latter consists of a colorless sirup and gives upon hydrolysis d-glucose and $\alpha$-glucoheptonic acid. The reaction is expressed as follows:

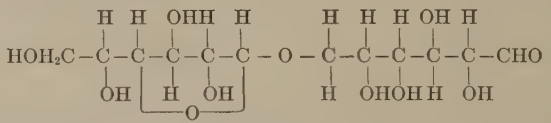
$$\underbrace{C_5H_{10}O_5CH-O-C_6H_{12}O_5COOH}_{\text{Maltose carboxylic acid}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\alpha\text{-Glucoheptonic acid.}}{C_6H_{13}O_6COOH}.$$

**Tests.** — Characteristic qualitative tests for maltose in presence of other sugars are lacking. The osazone reaction is one of the best

means of identification, the greater solubility of maltosazone affording an easy means for its separation from the less soluble osazones of other sugars; the influence of impurities in modifying the character of maltosazone must, however, always be borne in mind. The test has been modified by Grimbert* by treating the impure maltosazone with a little cold aqueous 50 per cent acetone and filtering; the maltosazone separates from the filtrate in pure crystalline form.

The inability of certain yeasts, as *Saccharomyces Marxianus* and yeast No. 538 of the Berlin Experimental Brewery, to ferment maltose is another means of separation and identification which may be employed under certain conditions.

**Configuration.** — The configuration of maltose has not been established with certainty. The following provisional formula suggested by Fischer answers, however, to most of the chemical properties and reactions of maltose:



**Synthesis of Maltose.** — Maltose has not been synthetized as yet with certainty by purely chemical means. The synthesis, however, seems to have been accomplished by the action of certain enzymes upon glucose in concentrated solution. Croft Hill† was the first to discover the synthetic action of enzymes; Hill observed, when extract of dried yeast, or takadiastase, was placed in concentrated glucose solutions, that a disaccharide sugar was formed. This sugar he believed at first to be maltose, and explained its formation by assuming the action of the enzyme to be reversible. Emmerling,‡ however, in repeating Hill's work, believed the disaccharide to be Fischer's isomaltose, and the same conclusion was also reached by E. F. Armstrong. Hill in a later work, while reaffirming his belief in the formation of some maltose, states that a different isomeric sugar, which he calls *revertose*, is the main product of the condensation.

*Armstrong Upon Enzymic Synthesis.* — By action of the enzyme *emulsin* upon d-glucose for a long period of time Armstrong§ observed the formation of a disaccharide which he believed to be mainly maltose. Emulsin itself does not hydrolyze maltose, and, according to Arm-

* J. Pharm. Chim. [6], **17**, 225.
† J. Chem. Soc., **73**, 634; **83**, 578.
‡ Ber., **34**, 600, 2206, 3810.
§ Proc. Roy. Soc. (1905), **76 B**, 592.

strong, in enzymic syntheses an isomeric sugar is obtained different from the one which the enzyme itself hydrolyzes. Thus:

| Sugar. | Enzyme. | Product of reaction. |
|---|---|---|
| { 1 maltose | + maltoglucase | = 2 d-glucose |
| { 2 d-glucose | + " | = 1 isomaltose |
| { 1 isomaltose | + emulsin | = 2 d-glucose |
| { 2 d-glucose | + " | = 1 maltose |

Armstrong is of the opinion that both maltose and isomaltose are formed by the action of concentrated hydrochloric acid upon glucose (see under isomaltose). The products of this condensation after neutralization were treated with emulsin, which hydrolyzed the isomaltose, and then with *Saccharomyces Marxianus,* which fermented the glucose but not the maltose. The disaccharide remaining in solution gave an osazone corresponding to that of maltose; this and the biological behavior of the sugar are strong indications of the formation of maltose. The question must be regarded, however, as unsettled until the sugar has been actually isolated in its pure crystalline form.

The hydrolyzing enzymes undoubtedly exercise a synthetic action in the living cell, but the conditions under which this is accomplished are not understood sufficiently as yet to enable the chemist to control the reaction in the laboratory.

**ISOMALTOSE,** $C_{12}H_{22}O_{11}$. — No other sugar has given rise to so much difference of opinion and uncertainty as isomaltose, a circumstance due to the fact that the so-called isomaltoses of different investigators are in all probability different compounds. The name isomaltose was first given by Fischer* to a synthetic disaccharide prepared as follows.

**Preparation.** — One hundred grams of d-glucose were dissolved in 400 gms. of cold fuming hydrochloric acid and the solution maintained for 15 hours at 10° to 15° C.; 4 kgs. of absolute alcohol were then added, the solution filtered from precipitated dextrins (reversion products) and the filtrate treated with a large excess of ether. The precipitate was filtered off, washed with alcohol and ether, pressed between filter paper, dissolved in a little water, the solution carefully neutralized with sodium carbonate, any alcohol and ether expelled by gentle warming and the excess of d-glucose removed by fermenting with yeast at 30° C. The unfermented residue (30 to 35 gms.) was dissolved in about 150 c.c. of water, exactly neutralized, and then heated with a solution of phenylhydrazine (30 gms.) in 50 per cent acetic acid (20 gms.) for $1\frac{1}{4}$ hours upon the water bath. The hot solution was then filtered from the slight

* Ber., **23,** 3687.

deposit of d-glucose-osazone; the filtrate upon cooling deposited crystals of isomaltose-osazone, which differed from maltose-osazone by its greater solubility in water and by its lower melting point, 150° C.

**Theories Regarding the Formation of Isomaltose.** — Armstrong, as previously mentioned, believes that maltose, as well as isomaltose, is formed in the above synthesis. The maltose by Fischer's method of purification is destroyed, however, by the action of the yeast.

Isomaltose was also obtained by Scheibler and Mittelmeier* by the action of strong acids upon starch, the glucose which is first formed being afterwards recondensed to form isomaltose and other reversion products. The isomaltose thus formed is no doubt similar to that of Fischer.

Isomaltose is also believed by Lintner,† Prior,‡ Albert§ and many other investigators to be formed during the diastatic conversion of starch. Opinions differ, however, as to whether this isomaltose is formed before or after maltose; the following schemes illustrate a few of the numerous theories which have been proposed in this connection:

Starch → amylodextrin → isomaltose → maltose.

Starch → amylodextrin ↗ isomaltose → maltose.
↘ maltodextrin → maltose.

Starch → amylodextrin . . . → maltose → d-glucose → isomaltose.

Lintner and Düll‖ prepared their isomaltose by saccharifying starch paste with malt extract at 70° C. The solution was then evaporated to a sirup, treated with strong alcohol, filtered from precipitated dextrin and the filtrate evaporated to expel alcohol; the d-glucose and maltose were then fermented away with yeast, the solution clarified with bone black, evaporated to a sirup, treated again with strong alcohol to precipitate remaining dextrins and the filtrate evaporated. In this manner a white amorphous hygroscopic residue was obtained, which corresponded to the formula and molecular weight of $C_{12}H_{22}O_{11} + H_2O$. The substance was easily soluble in water and 80 per cent alcohol, and showed in aqueous solution a specific rotation of $[\alpha]_D = +139$ to $+140$. The yield of isomaltose by this method was about 20 per cent the weight of starch. Lintner and Düll believe that the hydrolysis of starch consists in a change of amylodextrin, or soluble starch, into lower dextrins which are then transformed into isomaltose and the latter in turn into maltose.

* Ber., **24**, 301.
† Chem. Ztg., **16**, 15.
‡ Z. angew. Chem. (1892), 312, 872.
§ Chem. Centralbl. (1894), 1131.
‖ Ber., **26**, 2540.

Ling and Baker,* repeating the work of Lintner and Düll, obtained a residue which gave with phenylhydrazine a mixture of osazones, corresponding to d-glucose, maltose and an unknown trisaccharide. Ling and Baker also showed that a mixture of maltose and dextrin gave a crystallizable osazone which resembled in every way the so-called isomaltose-osazone.

Ost,† Ulrich,‡ Brown and Morris § and many other investigators also deny the formation of isomaltose during the diastatic conversion of starch and claim that the compound so designated is only a mixture of maltose with different dextrins. Lintner claims, however, that the isomaltose prepared by his method, although not absolutely pure, is sufficiently so to justify his conclusions as to its formation.

The views of Emmerling and Armstrong regarding the formation of isomaltose by action of maltoglucase upon glucose have already been mentioned. (See page 704.)

It is impossible to review in greater detail the copious literature upon isomaltose. No two authorities hold exactly the same opinion and the case is only an additional example of the lack of knowledge which still prevails regarding the different stages of starch conversion.

**Properties of Isomaltose.** — Isomaltose, as prepared by different investigators using different methods, shows certain differences in physical and chemical properties. All preparations of the sugar reduce Fehling's solution, Fischer's isomaltose having a reducing power 66 per cent and Lintner's 80 per cent of that of maltose. All preparations of the sugar upon heating with acids are hydrolyzed into d-glucose. Fischer's isomaltose is unfermented by yeast; that of Lintner in presence of considerable yeast is fermented but with considerable difficulty.

**Tests for Isomaltose.** — The osazone test for isomaltose is regarded as the most characteristic, the greater solubility and lower melting point distinguishing the osazone of isomaltose from that of maltose. The melting point of Fischer's isomaltose-osazone on rapid heating is 158° C. Ost, however, gives 145° C. The osazone of Lintner's isomaltose melts between 145° C. and 155° C. The osazone of both Fischer's and Lintner's isomaltose corresponds to the formula $C_{24}H_{32}N_4O_9$.

The fact that maltose in presence of impurities gives an osazone of the same melting point greatly lessens the value of the osazone test for isomaltose.‖

* J. Chem. Soc. Trans. (1895), **43**, 702, 739.
‡ Chem. Ztg., **19**, 1527.
† Chem. Ztg., **19**, 1504; **20**, 762.
§ Chem. News, **72**, 45.
‖ For fuller accounts of both maltose and isomaltose see Lippmann's "Chemie der Zuckerarten" and Sykes and Ling's "Principles and Practice of Brewing."

**LACTOSE.** — Milk sugar. Lactobiose.

$$C_{12}H_{22}O_{11} + H_2O.$$

**Occurrence.** — Lactose is a sugar of distinctly animal origin, no well-authenticated evidence as yet existing of its occurrence in the vegetable kingdom. The sugar is formed in the lacteal glands of all mammals and is built up from the glucose of the blood, although the manner in which this synthesis takes place is not understood. Lactose is found in milk in amounts varying from less than 1 per cent to over 12 per cent, according to the kind of animal, period of lactation and other factors. The percentage of lactose in the milk of different animals is given in the following table:

| Animal. | Lactose. | Animal. | Lactose. |
|---|---|---|---|
| | Per cent. | | Per cent. |
| Cow | 3.67–6.07 | Camel | 5.00–5.80 |
| Dog | 0.98–3.85 | Ass | 5.29–7.63 |
| Pig | 1.59–3.84 | Reindeer | 2.61–3.02 |
| Goat | 3.26–6.65 | Buffalo | 4.16–5.34 |
| Sheep | 3.43–6.62 | Elephant | 7.27–7.39 |
| Horse | 4.72–7.32 | Woman | 4.00–8.30 |

The percentage of lactose is usually less in colostrum than in normal milk. Pfeiffer* found in woman's milk just after birth 2.7 per cent lactose, at the end of one week 4 per cent, after 2 weeks 4.8 per cent, after 3 weeks 5.2 per cent, and after 5 months 6.5 per cent. Similar changes have been observed in the case of the cow and other animals.

When the secretion of milk is interfered with, as by interruption of nursing, or by some functional disorder, the lactose finds its way from the mammary glands into the blood and is then eliminated in the urine. Even in healthy cows, just prior to calving, lactose has been found in the urine to the extent of 0.5 per cent.

**Preparation of Lactose.** — Lactose is manufactured commercially from the whey of cheese factories. The curd, which is precipitated from milk by means of rennet, and which contains the casein and fat, is filtered off and made into cheese. The filtrate from the curd is the whey and contains upon the average about 93.50 per cent water, 4.80 per cent lactose, 1.00 per cent proteids, 0.50 per cent ash and 0.20 per cent fat. For the manufacture† of milk sugar the whey is heated to 75° to 85° C. and then treated with 6 to 10 per cent of milk

* Chem. Ztg. **18,** 1543.

† F. Fischer's "Handbuch der chem. Technologie" (1902), II, 282.

of lime at 20 degrees Bé. The free lactic acid is thus neutralized, insoluble calcium phosphate is formed, albuminoids are coagulated and fat and other suspended impurities mechanically precipitated. The precipitate is filtered off and the filtrate saturated with carbon dioxide, filtered, evaporated, boiled to grain and centrifuged in the same manner as for beet sugar manufacture. The yield of crystallized milk sugar by this method is about 3.4 per cent of the whey.

**Properties of Lactose.** — Milk sugar, as ordinarily prepared, consists of large rhombic hemihedral crystals corresponding to the formula $C_{12}H_{22}O_{11} + H_2O$. The water of crystallization is given up with considerable difficulty. The best method of dehydration is to precipitate a hot concentrated aqueous solution with 5 volumes of absolute alcohol. The fine crystalline powder thus obtained is dried first at 100° C., and then at 127° to 130° C , when the last traces of water are given off without change in color or other evidences of decomposition.

Lactose hydrate is soluble in 5.87 parts of water at 10° C. and 2.5 parts of water at 100° C.; it easily forms supersaturated solutions. The sugar is insoluble in absolute ethyl and methyl alcohols and in ether. The presence of free alkali, and of different salts of the alkalies, increases the solubility of lactose in much the same manner as with sucrose.

Lactose hydrate is dextrorotatory, $[\alpha]_D = +52.50$ which value is practically constant for concentrations up to $c = 36$. The influence of temperature upon the $[\alpha]_D$ of lactose has already been referred to.

The specific rotation of lactose anhydride is +55.3. (The same value is obtained by calculation from the $[\alpha]_D$ of the hydrate, $+52.50 \times \frac{360}{342}$.)

Freshly prepared solutions of lactose hydrate exhibit mutarotation. Tollens and Parcus* found for a solution of 4.841 gms. lactose hydrate dissolved to 100 c.c. the following values:

| Time after solution. | Specific rotation. | Time after solution. | Specific rotation. |
|---|---|---|---|
| Minutes. | | Hours. | |
| 8 | +82.91 | 2 | 62.17 |
| 10 | 82.52 | 4¼ | 54.32 |
| 20 | 79.69 | 6 | 53.43 |
| 45 | 73.26 | 24 (constant) | 52.53 |
| 60 | 70.04 | | |

Upon boiling the solution or adding 0.1 per cent ammonium hydroxide the mutarotation is destroyed almost immediately. Addition of mineral acids accelerates the change to constant rotation.

* Ann., **257**, 170.

*Low-Rotating Modification of Lactose.* — Upon rapidly evaporating a solution of 2 to 3 gms. of ordinary lactose hydrate in 10 c.c. of water in a platinum dish and drying the residue at 98° C., Schmöger* obtained a form of lactose which showed after solution a rotation of only + 34.4 (calculated to $C_{12}H_{22}O_{11} + H_2O$) but after 24 hours' standing increased to the normal constant value of +52.5. Erdmann† obtained the same form of lactose by rapidly boiling down a solution of lactose hydrate in a metal dish until the supersaturated solution suddenly solidified to a porous mass of small water-free crystals. Tanret‡ obtained the low-rotating milk sugar (his so-called $\gamma$ modification) by rapidly evaporating a solution of ordinary lactose (Tanret's so-called $\alpha$ modification) at 108° C., drying the crystals over concentrated sulphuric acid, dissolving rapidly in 3 parts of water and precipitating at once with a large excess of alcohol. This process, after repeating several times, gives the pure $\gamma$ modification in the form of water-free crystals, soluble in 2.2 parts of water at 15° C., which solution, however, on standing deposits crystals of the ordinary hydrate. Tanret's $\gamma$ milk sugar 5 minutes after solution gave $[\alpha]_D = +34.5$, which value slowly increased upon standing to that of constant rotation. The change was effected immediately by adding a trace of alkali.

*Tanret's β-lactose.* — Tanret obtained an apparent third modification of lactose by allowing a concentrated solution of the hydrate to crystallize at exactly 85° to 86° C., or by treating the warm solution with 3 to 4 times its amount of absolute alcohol. Crystals were obtained, corresponding to the formula $C_{12}H_{22}O_{11} + \frac{1}{2} H_2O$, which gave immediately after solution in water the constant rotation +55.3 (calculated to the anhydride $C_{12}H_{22}O_{11}$).

*Hudson Upon the Modifications of Lactose.* — Hudson§ in a recent study of lactose and its modifications came to the conclusion that Tanret's so-called $\beta$ or constant rotating lactose was not a definite compound but a mechanical mixture of the high and low rotating forms. The same conclusion was arrived at by Roux‖ and also by Trey.¶ Hudson, Roux, and Trey all confirm the earlier observation of Erdmann that the velocities of mutarotation for the high and low rotating forms of lactose are the same in value, and draw from this the conclusion that the two changes in rotation are only opposite parts of one balanced reaction, which in its ultimate form may be expressed by the equation $\alpha$-lactose $\rightleftarrows$ $\beta$-lactose. (The symbol $\beta$ is given at present to

* Ber., **13,** 1915.
† Ber., **13,** 2180.
‡ Bull. soc. chim. [3], **13,** 625.
§ Z. physik. Chem., **44,** 487.
‖ Ann. chim. phys. [7], **30,** 422 (1906).
¶ Z. physik. Chem., **46,** 620.

the low rotating modifications of all sugars instead of $\gamma$ as first used by Tanret.) This identity in mutarotation for the high and low rotating forms of lactose is shown by the following results of Hudson: *

TABLE CI

*Mutarotation at 0° C. of Lactose Hydrate and β-Lactose Anhydride*

| Time in hours. | $[\alpha]_D$ calculated for $C_{12}H_{22}O_{11}$. | | Velocity of mutarotation, $\frac{1}{t}\log_{10}\frac{r_0-r_\infty}{r-r_\infty}$. | |
|---|---|---|---|---|
| | Ordinary lactose. | β-Lactose. | Ordinary lactose. | β-Lactose. |
| 0 | +89.13 | +39.6 | ..... | ..... |
| 2 | +84.96 | +41.6 | 0.0284 | 0.0298 |
| 4 | +80.79 | +43.4 | 0.0306 | 0.0306 |
| 6 | +77.48 | +44.9 | 0.0299 | 0.0300 |
| 8 | +74.45 | +46.3 | 0.0307 | 0.0305 |
| 10 | +72.06 | +47.6 | 0.0303 | 0.0312 |
| ∞ | +55.16 | +55.2 | ..... | ..... |

The anhydrous low rotating β-lactose was obtained by Hudson in large crystals by slowly evaporating a solution of ordinary lactose hydrate at from 95° C. to 100° C. The transition temperature above which the β-lactose separates was found by Hudson† to be 93° C. These two forms of lactose differ greatly in solubility and other physical properties as well as in optical activity. Thus the final solubility (calculated to $C_{12}H_{22}O_{11}$) in water at 0° C. was found by Hudson to be 10.6 per cent for ordinary lactose and 42.9 per cent for the β-lactose. The much greater solubility of the β-lactose has rendered this form of milk sugar especially suitable for certain medicinal and other purposes.

**Reactions of Lactose.** — Upon heating at 170° to 180° C. lactose is converted into lacto-caramel, a dark brown substance which resembles in many of its properties the caramel of sucrose.

Lactose reduces Fehling's solution about 70 per cent as strongly as d-glucose. If, after the end of the reduction, the solution be filtered from cuprous oxide, the filtrate be weakly acidified with hydrochloric acid and again boiled with Fehling's solution, a second quantity of copper is reduced‡ and in about one-half the original amount. The cause of the phenomenon, which is similar to that noted for maltose, has not been fully explained. Barfoed's copper acetate solution is not reduced by lactose.

By action of sodium amalgam lactose is broken up and reduced with formation of mannite, dulcite, lactic acid and other decomposition products.

* J. Am. Chem. Soc., **26**, 1065. † J. Am. Chem. Soc., **30**, 1767.

‡ Herzfeld, Ann., **220**, 200.

By action of bromine in aqueous solution lactose is oxidized to lactobionic acid. The latter was first obtained by Fischer and Meyer,* as a colorless sirup soluble in water, less soluble in alcohol but insoluble in ether. Lactobionic acid is hydrolyzed by dilute acids into d-galactose and d-gluconic acid. This reaction and the reducing properties of lactose indicate the presence of a free aldehyde group attached to a glucose residue. The oxidation of lactobionic acid is explained as follows:

$$\underbrace{\underset{\text{d-Galactose radical}}{C_5H_{10}O_5CH-O-}\underset{\text{d-Glucose radical}}{C_5H_{10}O_4CHO}}_{\text{Lactose}} + O = \underbrace{\underset{\text{d-Galactose radical}}{C_5H_{10}O_5CH-O-}\underset{\text{d-Gluconic acid radical}}{C_5H_{10}O_4COOH}}_{\text{Lactobionic acid.}}$$

Lactobionic acid upon oxidation with hydrogen peroxide in presence of basic ferric acetate is degraded to galactoarabinose (p. 644).

Oxidation of lactose with nitric acid produces d-saccharic acid by oxidation of the d-glucose radical, and mucic acid by oxidation of the d-galactose radical. The discovery of mucic acid as a result of this oxidation was made by Scheele in 1780. The yield of mucic acid from lactose by the following method of Kent and Tollens† is about 40 per cent:

100 gms. of pulverized lactose are dissolved in 1200 gms. nitric acid of 1.15 sp. gr.; the solution is evaporated to between 150 c.c. and 200 c.c. upon the water bath and, after cooling, 200 c.c. of water added. The crystals of mucic acid are filtered off after 24 hours' standing.

*Action of Alkalies.* — Lactose upon heating with dilute alkalies‡ undergoes an almost complete loss of optical activity, the sugar molecule undergoing partial hydrolysis and rearrangement with formation of d-galactose, pseudotagatose and other products of unknown composition. Upon heating with concentrated alkalies lactose solutions are colored brown, the lactose being broken up into lower decomposition products among which lactic acid occurs in greatest amount. Lactose exposed to the action of alkalies in the sunlight yields according to Duclaux over 50 per cent lactic acid.

Lactose upon long treatment with calcium hydrate is converted into isosaccharinic acid. The reaction according to Kiliani § is best conducted as follows: A cold solution of 1 kg. milk sugar in 9 liters of water is treated with 450 gms. calcium hydroxide and allowed to stand

* Ber., **22,** 361.

† Z. Ver. Deut. Zuckerind., **35,** 38.

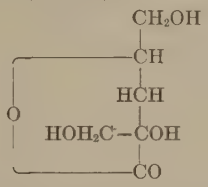
‡ Lobry de Bruyn and van Ekenstein, Rec. Trav. Pays-Bas, **14,** 156, 203.

§ Ber., **16,** 2625; **18,** 631.

at room temperature with frequent shaking for 5 to 6 weeks. The clear brown solution is saturated with carbon dioxide, filtered from calcium carbonate, heated to boiling, again filtered and concentrated to 2 liters. Calcium isosaccharinate crystallizes out and calcium metasaccharinate remains in solution. The precipitate of the former is filtered off, washed with cold water and dried. A weighed quantity of the salt is then dissolved in water and decomposed with an exact amount of oxalic acid. The filtered solution contains free isosaccharinic acid, $C_6H_{12}O_6$, which upon evaporation splits off water, and crystallizes out as its lactone or isosaccharin, $C_6H_{10}O_5$.

*Isosaccharin* consists of beautiful double-refracting crystals, easily soluble in water, alcohol and ether. It melts at 95° C. and sublimes without decomposition. It is dextrorotatory ($[\alpha]_D = +63$), is not fermented by yeast and does not reduce Fehling's solution.

Kiliani's isosaccharin is identical with Nef's $\alpha$-isosaccharin, obtained by the action of sodium hydroxide upon d-galactose (p. 603), and has the following structural formula:



**Hydrolysis of Lactose by Acids.** — Lactose upon heating on a boiling water bath with 4 parts of 2 per cent sulphuric acid is hydrolyzed * into equal parts of d-glucose and d-galactose:

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{d-Galactose.}}{C_6H_{12}O_6}.$$

The hydrolysis may also be carried out with hydrochloric acid, the reaction proceeding at ordinary temperature with sufficient concentration of acid. Urech † found that a solution containing 12 gms. lactose and 32 gms. hydrochloric acid to 100 c.c. was hydrolyzed almost completely after 12 hours' standing at 23° C. The hydrolysis of lactose by means of acids proceeds with much greater difficulty than that of sucrose or maltose. As in the case of maltose the prolonged action of acid causes a slight loss of reducing sugar due to formation of levulinic acid, humus substances, reversion products, etc.

**Fermentation of Lactose.** — In so far as the different yeasts, bacteria, moulds, etc., contain the lactose-splitting enzyme *lactase*, they ferment lactose in the same manner as d-glucose and d-galactose.

* Ost, Ber., **23**, 3006. † Ber., **18**, 3048.

Many organisms which ferment d-glucose and d-galactose are without action upon lactose, and in such cases it is generally supposed that lactase is absent. But whether in all lactose fermentations the sugar must first be hydrolyzed by a lactase is uncertain, although many authorities at present incline to this opinion.

Ordinary baker's and brewer's yeasts exercise no pronounced fermentation upon lactose. Hansen,* in fact, found that none of the common alcohol-producing yeasts in pure culture was able to ferment lactose. Invertase, maltase, zymase and the other enzymes of ordinary yeast have in fact no action upon lactose. The action of different yeasts upon lactose and other sugars is given by Fischer† and Thierfelder in Table CII.

TABLE CII

*Showing Action of Yeasts Upon Different Sugars*

| Kind of yeast. | d-Mannose. | d-Fructose. | d-Galactose. | d-Talose. | l-Mannose. | l-Gulose. | Sorbose. | l-Arabinose. | Rhamnose. | α-Glucoheptose. | α-Glucooctose. | Sucrose. | Maltose. | Lactose. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. Pastorianus I | ∧∧∧ | ∧∧∧ | ∧∧∧ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. Pastorianus II | ∧∧∧ | ∧∧∧ | ∧∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. Pastorianus III | ∧∧∧ | ∧∧∧ | ∧∧∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. cerevisiæ I | ∧∧∧ | ∧∧∧ | ∧∧∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. ellipsoideus I | ∧∧∧ | ∧∧∧ | ∧∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. ellipsoideus II | ∧∧∧ | ∧∧∧ | ∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. Marxianus | ∧∧∧ | ∧∧∧ | ∧∧∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | 0 | 0 |
| S. membranæfaciens | 0 | 0 | 0 | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brewery yeast | ∧∧∧ | ∧∧∧ | ∧∧∧ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| Distillery yeast | ∧∧∧ | ∧∧∧ | ∧ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | ∧∧∧ | 0 |
| S. productivus | ∧∧∧ | ∧∧∧ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | .. | 0 | ∧ | ∧∧∧ | 0 |
| Milk-sugar yeast | ∧∧ | ∧∧∧ | ∧ | 0 | 0 | .. | 0 | 0 | 0 | 0 | 0 | ∧∧∧ | 0 | ∧∧∧ |

∧∧∧ =complete fermentation after 8 days.
∧∧ =almost complete fermentation after 8 days.
∧ =only partial fermentation after 8 days.
0 =no fermentation after 8 days.

A large number of so-called "milk-sugar yeasts" have been isolated from the products of cheese factories and from such milk beverages as Kumiss, Kefir, Mazun, etc. It is a disputed question, however, whether these so-called yeasts belong to the *Saccharomyces* or to the *Torulaceæ*. The latter ferment lactose with production of alcohol‡ and according to Beyerinck§ contain considerable amounts of the enzyme lactase.

* Chem. Centralbl. (1888), 1209.
† Ber., **27**, 2031.
‡ Adametz, Chem. Centralbl. (1889), 260.
§ Chem. Centralbl. (1897), II, 1012.

*Lactase.* — Lactase is best prepared according to Fischer* by washing Kefir corns (the granular concretions of yeasts and bacteria which constitute the Kefir ferment) with water, grinding the air-dried mass with pulverized glass and extracting with water. Lactase is much less sensitive to the paralyzing action of alcohol, acids, etc., than invertase, amylase, maltase and other enzymes.

In addition to its occurrence in the Torulaceæ and other organisms lactase is found in several species of moulds and fungi and also in higher plants. The crude emulsin, obtained by extracting almonds and the seeds of other Rosaceæ, contains a lactase, although the emulsin from *Aspergillus niger*, the cherry laurel and several other sources has no hydrolyzing action upon lactose.

Lactases are very abundant in the animal kingdom and this would be expected from the importance which lactose plays in animal nutrition, especially during the nursing period. Lactases have been found in the mucous membranes of the stomach and intestines of newly born infants and in the same tissues of the young of most mammals. Lactase also occurs in the membranes of the digestive tract of older animals and is found in preparations of such enzymes as ptyalin, pepsin, trypsin, rennet, etc. It is a significant fact that the secretion of lactase is intensified by increasing the amount of milk or milk sugar in the food.

*Lactic and Butyric Fermentations.* — Lactose is more easily subject to the lactic and butyric fermentations than any other sugar. In fact, nearly all the organisms which produce lactic acid from d-glucose and sucrose ferment lactose in the same way. On the other hand, there are a very large number of organisms which produce lactic acid from lactose but not from sucrose or glucose.

The lactic fermentation to secure best results must be conducted in presence of suitable nutrients and also in presence of calcium carbonate (or similar substance) to neutralize the free acid as soon as formed. The presence of much free acid checks the fermentation, so that in milk, for example, the percentage of free lactic acid under ordinary conditions never exceeds 0.5 to 0.6 per cent. In presence of sufficient calcium carbonate the fermentation of lactose into lactic acid under favorable conditions may be almost quantitative.

The various lactic organisms differ greatly in their action upon lactose. While the ordinary optimum temperature is about 30° C., some bacteria produce fermentation as low as 10° C., and others as high as 52° C. Some lactic organisms are retarded and others stimulated by the influence of atmospheric oxygen. The lactic bacteria also

* Ber., **27**, 3479.

differ as to the kind of lactic acid which they produce, some organisms form d-lactic acid, others l-lactic acid, others d, l-lactic acid and others mixtures of d- and l-lactic acids in varying amounts.

The literature upon the lactic acid bacteria is exceedingly copious and for a review of the subject reference should be made to the works of Lafar,* Jörgensen,† Emmerling,‡ and others.

In the butyric fermentation of lactose the butyric acid may be formed either primarily from the lactose or secondarily from the lactic acid produced by the lactic fermentation. A large class of both aërobic and anaërobic bacteria ferment lactose with production of butyric acid, but the action of only a few of these has been studied. In addition to butyric acid, formic, acetic, propionic, valeric and succinic acids, butyl alcohol, acetone, carbon dioxide, hydrogen and methane have been found among the products of different butyric fermentations.

A large number of organisms ferment lactose with formation of a viscous gum. The bacteria of this class have been especially studied in connection with the slimy or ropy fermentation of milk. The *Leuconostoc mesenterioides* and many other organisms which form dextran from sucrose do not exercise this action upon lactose.

**Compounds of Lactose.** — Lactose, the same as maltose, contains a reactive aldehyde group and forms hydrazones, osazones, methyl lactosides, ureides, mercaptals, etc., in the same manner as other reducing sugars. The most important of these compounds from the analytical standpoint is lactose-phenylosazone, $C_{12}H_{20}O_9(:N-NHC_6H_5)_2$, which was first prepared by Fischer§ by heating 1 part lactose, $1\frac{1}{2}$ parts phenylhydrazine chloride, 2 parts sodium acetate and 30 parts water for $1\frac{1}{2}$ hours. The osazone separates from the cold solution in the form of yellow needles, which after recrystallizing melt at 200° C. Lactose phenylosazone is soluble in 80 to 90 parts boiling water, in hot alcohol and in hot glacial acetic acid; it is insoluble in ether, benzol and chloroform.

Lactose forms with nitric acid, in presence of ice-cold strong sulphuric acid, tri-, tetra-, penta,- hexa- and octonitrates; and with acetic anhydride mono-, di-, tetra-, hexa- and octacetates. The octacetate is the best known acetate, and is obtained‖ by heating 5 gms. lactose with 20 gms. acetic anhydride and 5 gms. water-free sodium

* "Technische Mykologie."

† "Microorganismen der Gärungsindustrie."

‡ "Zersetzung Stickstoffreier organ. Substanzen durch Bacterien," Braunschweig (1902), pp. 25–84.

§ Ber., **17,** 579; **20,** 821.

‖ Schmöger, Ber., **25,** 1452.

acetate just to boiling and then pouring the mass into water. The precipitated compound is recrystallized from alcohol. Lactose octacetate consists of white crystals of the formula $C_{12}H_{14}(C_2H_3O)_8O_{11}$, and melting according to different observers between 86° C. and 106° C. The variation in melting point is attributed by some chemists to the existence of two isomers. The rotation of the carefully purified compound is given by Schmöger as $[\alpha]_D = -3.5°$ (in chloroform).

Lactose forms with alkalies and alkaline earths a number of lactosates, none of which, however, has any analytical or technical importance.

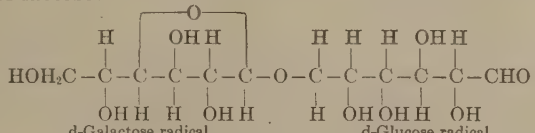
Upon treatment with hydrocyanic acid lactose forms a nitrile, which yields after saponification lactose carboxylic acid; the latter consists of a colorless sirup and gives upon hydrolysis d-galactose and $\alpha$-glucoheptonic acid.

$$\underbrace{C_5H_{10}O_5CH-O-C_6H_{12}O_5COOH}_{\text{Lactose carboxylic acid}} + H_2O = \underbrace{C_6H_{12}O_6}_{\text{d-Galactose}} + \underbrace{C_6H_{13}O_6 \cdot COOH}_{\alpha\text{-Glucoheptonic acid.}}$$

**Tests for Lactose.** — Characteristic qualitative tests for lactose in presence of other sugars are wanting. The formation of mucic acid upon oxidation with nitric acid is a valuable confirmatory test; although it must always be borne in mind that the same reaction is also given by d- and l-galactose, galactonic acid and galactan. Separation of lactose osazone, after careful recrystallization and purification, offers a fairly reliable means of identification. In case several reducing sugars are present, the mixture of osazones should be heated with boiling water and filtered; the osazones of lactose, maltose and other disaccharides will be found in the filtrate, from which crystallization takes place upon cooling.

In case of mixtures, the destruction of d-glucose, d-fructose, d-mannose, sucrose, maltose and other fermentable sugars by means of pure cultures of yeasts which do not ferment lactose (Table CII) may be employed to advantage before making tests for lactose.

**Configuration.** — The configuration of lactose has not been established with certainty. The following constitutional formula proposed by Fischer* answers, however, to most of the chemical properties and reactions of lactose:




* "Untersuchungen über Kohlenhydrate" (1909), p. 92.

**Synthesis.** — The synthesis of lactose has not yet been accomplished either chemically or by means of enzymes.

**Isolactose.** — $C_{12}H_{22}O_{11}$.

Isolactose has not been found in nature. The name was given by Fischer and Armstrong* to a disaccharide, which they obtained through the synthetic action of Kefir lactase upon a concentrated solution of equal parts d-glucose and d-galactose.

Fifty grams of finely shredded Kefir corns were shaken up with 300 c.c. water and 5 c.c. toluol for 48 hours at ordinary temperature; 200 c.c. of the extract, 100 gms. each of d-glucose and d-galactose, and 10 c.c. of toluol were placed in a closed flask and allowed to stand 15 days at 35° C. After diluting with 1 volume of water, boiling 10 minutes and cooling, the filtered solution was fermented with top yeast to remove the residual d-glucose and d-galactose. The isolactose remaining in solution was separated only in form of isolactose-phenylosazone, $C_{24}H_{32}N_4O_9$, which consisted of fine needles melting at 190° to 193° C.

**Trehalose.** — Trehabiose. Mycose. Mushroom sugar.

$$C_{12}H_{22}O_{11} + 2\,H_2O.$$

**Occurrence.** — Trehalose is a disaccharide discovered by Wiggers† in ergot (the sclerotium growth produced by the fungus *Claviceps purpurea* upon rye and other grasses), and later found to occur in nearly all fungi and mushrooms. Muntz‡ found in the dry substance of *Agaricus muscarius*, *A. sulfureus*, *A. sambucinus*, *Fungus sambuci* and *Boletus cyanescens* as much as 10 per cent trehalose. Bourquelot§ also found trehalose in varying amounts in *Sclerotinia tuberosa*, *Phallus impudicus*, *Boletus satanas* and in 36 other varieties of *Boletus*, *Amanita*, *Pholiota*, *Hypholoma* and *Lactarius*. Trehalose is unequally distributed through the several tissues of mushrooms; the stems of *Boletus edulis*, for example, were found by Bourquelot to contain 2.45 per cent trehalose, the caps 1.38 per cent and the pores none at all. The quantity of trehalose also varies according to the period of vegetation, being greatest just before the formation of spores. After the mushroom is picked, the trehalose is rapidly hydrolyzed by the enzyme *trehalase* into d-glucose, the latter being afterwards reduced through some unknown biological process to mannite.

*Trehala-manna and Trehalum.* — Trehalose was also found by Berthelot‖ in the so-called Trehala-manna, a drug long used in the

* Ber., **35**, 3144.
† Ann., **1**, 173 (1832).
‡ Compt. rend., **79**, 1182.
§ Compt. rend., **108**, 568; **111**, 578; **117**, 826.
‖ Ann. chim. phys. [3], **55**, 272.

Orient, and consisting of the cocoon or gall-like concretions formed by a species of beetle upon certain spiny plants of Syria and Persia. Trehala-manna also contains a polysaccharide *trehalum*, discovered by Guibourt* and later investigated by Scheibler and Mittelmeier.† Trehala-manna is first extracted with successive quantities of boiling alcohol to remove the trehalose and then with boiling water. The hot-water extract is filtered through a hot-water funnel and the trehalum separates from the cold filtrate as a white tasteless crystalline powder, with a composition corresponding to the provisional formula $C_{24}H_{42}O_{21}$. Trehalum is soluble in 56 parts of boiling water, is dextrorotatory ($[\alpha]_D = +179$), and is hydrolyzed by hydrochloric or sulphuric acids into d-glucose; it is not reducing and forms no compound with phenylhydrazine. Solid trehalum is colored by alcoholic iodine solution violet and solutions of trehalum wine red. Trehalum has a certain resemblance to starch and it possibly bears the same relation to trehalose that starch bears to maltose.

**Preparation of Trehalose.** — Finely shredded trehala-manna or mushrooms (freshly picked) are boiled with strong alcohol and the filtered extract set aside to cool. Crystals of trehalose will usually separate immediately; if crystallization does not take place the alcoholic extract is concentrated by evaporation and again set aside.

**Properties.** — Trehalose, as ordinarily prepared, is obtained in the form of large colorless rhombic crystals having the formula $C_{12}H_{22}O_{11} + 2\ H_2O$. The crystals have a sweet taste, are soluble in 1.7 parts of water and in 100 parts of hot alcohol, but are insoluble in ether. The hydrate begins to melt in its water of crystallization at 94° C., and liquefaction is complete between 96.5° C. and 97.5° C. The water of crystallization is lost at about 130° C. and the trehalose anhydride thus obtained melts at about 200° C.

Trehalose is strongly dextrorotatory; Schukow‡ found for the hydrate ($c = 7.282$) $[\alpha]_D^{20} = +178.3$, from which the calculated value for the anhydride would be $[\alpha]_D^{20} = +197.1$. Apping§ determined for the anhydride $[\alpha]_D = +197.28$. Mutarotation is not observed.

**Reactions.** — Trehalose, like sucrose, does not reduce Fehling's solution. The absence of aldehyde or ketone properties is also shown by the failure of trehalose to form hydrazones or osazones, by its resistance towards solutions of boiling alkalies, and by the non-formation of acid oxidation products in aqueous solution by means of bromine at ordinary temperature. Upon warming with dilute nitric acid

* Compt. rend., **46**, 1213.
† Ber., **26**, 1331.
‡ Z. Ver. Deut. Zuckerind, **50**, 818.
§ Dissertation, Dorpat., 1885.

trehalose is oxidized to d-saccharic acid and by strong nitric acid to oxalic acid.

Upon heating with dilute hydrochloric or sulphuric acids for several hours, trehalose is hydrolyzed into 2 molecules of d-glucose.

$$\underset{\text{Trehalose}}{C_{12}H_{22}O_{11}} + H_2O = 2\,\underset{\text{d-Glucose.}}{C_6H_{12}O_6}.$$

The hydrolysis is accomplished only with considerable difficulty. According to Winterstein * 6 hours' boiling with 5 per cent sulphuric acid is necessary to secure complete conversion; the yield of d-glucose obtained at the end of this time was 99.45 per cent of the theoretical.

Trehalose forms a number of acetates upon heating with acetic anhydride and a number of benzoates upon treatment with benzoyl chloride. Compounds of trehalose with calcium, strontium and lead have also been prepared.

**Fermentation.** — Trehalose is not readily fermented by ordinary baker's or brewer's yeast. The sugar is fermented, however, by a number of wild yeasts; according to Dubourg † a large number of yeasts can ferment trehalose after special methods of cultivation and adaptation. A number of moulds, as *Mucor mucedo*, ferment trehalose in absence of air with production of alcohol.

The fermentation of trehalose is apparently conditioned by the presence of a special hydrolyzing enzyme *trehalase*, which was first isolated by Bourquelot ‡ from a culture of *Aspergillus niger* at the time of spore formation. The mould was distintegrated by grinding with sand, dehydrated by washing with 95 per cent alcohol and then dried in a vacuum at low temperature. The dried mass was then extracted with water, and the trehalase precipitated from the filtered extract by strong alcohol. The purified enzyme consisted of a white amorphous substance, easily soluble in water and having below 53° C. a strong hydrolytic action. Its activity was destroyed by heating to 63° C.

Trehalase is found, according to Bourquelot, in other moulds and in higher fungi, such as *Morchella* and *Polyporus*. It has also been detected in green malt. In the animal kingdom its presence has been reported in human urine, in the pancreas and intestines of rabbits, in the intestines of calves, horses, etc., and in the blood serum of certain fishes.

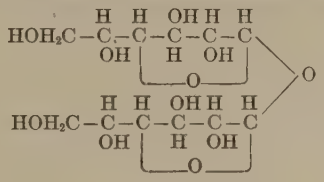
Emulsin, invertase, diastase and ptyalin have no hydrolytic action upon trehalose.

**Tests.** — No reliable qualitative tests are known for the detection of trehalose in mixture with other sugars. According to Bourquelot, if

* Ber., **26,** 3094. † Compt. rend., **128,** 440. ‡ Compt. rend., **117,** 826.

a glass plate be gently rubbed with a crystal of trehalose and a drop of sirup containing trehalose be spread over the rubbed area, crystallization will develop and the lines of contact, where the trehalose crystal touched the glass, will appear visible.

**Configuration.** — The constitutional formula of trehalose has not been established. Owing to the absence of reducing properties, it is supposed that the aldehyde groups of the two glucose radicals are combined together. The following arrangement has been proposed:



**MELIBIOSE.** — $C_{12}H_{22}O_{11} + 2\,H_2O$.

**Occurrence.** — This disaccharide has not been found in nature as yet in the free condition. It was first prepared by Scheibler and Mittelmeier* by a partial hydrolysis of the trisaccharide raffinose by means of mineral acids (see page 737).

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}}.$$

The same hydrolysis is effected by means of invertase. A pure culture of top-fermentation yeast will hydrolyze raffinose into melibiose and d-fructose and, after fermenting the latter, leave a solution containing melibiose. Bottom-fermentation yeast cannot be used owing to the occurrence of an enzyme, *melibiase*, which hydrolyzes melibiose as fast as it is formed.

**Preparation.** — For the preparation of melibiose either of the methods proposed by Bau† may be used.

*Preparation of Melibiose from Raffinose by Fermentation.* — A sterilized solution, containing 20 gms. raffinose in 250 c.c. of water, is fermented with 30 gms. of a pressed pure culture of Frohberg top-fermentation yeast at 31° C. for one day. The solution is filtered, sterilized and again fermented with 10 gms. of yeast at 31° C. for several days. The filtered solution, after decolorizing by means of bone black, is evaporated, the hot sirup poured into hot 95 per cent alcohol, and the cold solution, after decanting from precipitated impurities, treated with an excess of ether. The impure melibiose, which is thus precipitated,

* Ber., **22**, 1678; **23**, 1438. † Chem. Ztg., **26**, 69.

is dissolved in 70 per cent alcohol and precipitated again at 0° C. as its barium compound by adding cold aqueous barium hydroxide solution and cold 92 per cent alcohol. The barium melibiate is filtered off, washed with cold alcohol, pressed, suspended in water and decomposed by means of carbon dioxide. Any barium remaining in solution is precipitated by adding the exact amount of dilute sulphuric acid (avoiding even the slightest excess) and the filtrate evaporated below 80° C. to a sirup; 92 per cent alcohol is added until the alcoholic strength of the diluted sirup is 70 per cent and ether is added just beyond the point of precipitation. The ether-alcohol solution after filtration is set aside and gradually deposits crystals of melibiose, which may be purified by recrystallizing from 78 per cent alcohol.

*Preparation of Melibiose from Raffinose by Hydrolysis with Acid.* — A 10 to 20 per cent solution of raffinose is boiled with 2 per cent acetic acid; the solution is concentrated in a porcelain dish to a thick sirup which after cooling is rubbed up with 2 volumes of 95 per cent alcohol. The alcoholic solution is decanted and ether added with shaking to the point of permanent turbidity; the solution after standing 2 days is filtered from the precipitate and allowed to stand for several weeks in a closed flask. Crystallization gradually takes place; the process may be hastened by priming with a little melibiose from a previous preparation. The crude melibiose thus obtained is purified by recrystallizing from alcohol.

**Properties.** — Melibiose is obtained from aqueous solution in the form of monoclinic crystals having the formula $C_{12}H_{22}O_{11} + 2\ H_2O$. The sugar has a mild sweet taste, and when warmed begins to melt in its water of crystallization at 75° to 80° C. Upon heating in a thin layer gradually to 110° C. all water is expelled. The anhydride is exceedingly hygroscopic and reabsorbs water from the air with great avidity. One gram of crystallized melibiose is dissolved by 0.4186 gms. of water at 17.5°C., by 6.8137 gms. of methyl alcohol and by 175.67 gms. of ethyl alcohol.

Melibiose is strongly dextrorotatory. Bau found for the hydrate $[\alpha]_D^{20} = +\ 129.50$, from which the value for the anhydride $= +\ 143$. The sugar exhibits mutarotation, the initial value for $[\alpha]_D$ being less than the constant reading ($[\alpha]_D^{20}$ after 5 minutes $= +\ 108.68$).

**Reactions.** — Melibiose reduces Fehling's solution, the reducing power as $C_{12}H_{22}O_{11}$ being about 95 per cent that of maltose. Reduction with sodium amalgam gives a complex alcohol, *melibiotite*, $C_{12}H_{24}O_{11}$, which consists of an easily soluble non-reducing sirup, and is hydrolyzed by acids into d-galactose and d-mannite. Upon heating with hydrochloric

or sulphuric acid melibiose is slowly hydrolyzed into d-glucose and d-galactose.

$$\underset{\text{Melibiose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{d-Galactose.}}{C_6H_{12}O_6}.$$

Melibiose is thus seen to yield the same products of hydrolysis as lactose. Hydrolysis is not effected by acetic, tartaric, citric or lactic acids. Melibiose is rapidly hydrolyzed by the enzyme melibiase, and also, but more slowly, by emulsin.

Owing to the presence of a reactive aldehyde group melibiose forms a considerable number of hydrazones and osazones. The sugar also forms an octacetate upon boiling with acetic anhydride and sodium acetate. The compound consists of needle-shaped crystals with very bitter taste, having the formula $C_{12}H_{14}(C_2H_3O)_8O_{11}$, only slightly soluble in water, soluble in alcohol, chloroform and benzol and showing dextrorotation ($[\alpha]_D = +94.2$.)

**Fermentation.**—Melibiose is readily fermented into alcohol and carbon dioxide by all bottom-fermentation yeasts, but not by pure cultures of the ordinary top yeasts. Certain exceptions to the latter rule have been noted in a few cases, but fermentation in these instances may have been due to contamination or to the unexplained phenomenon of transition by which a bottom yeast acquires top-fermentation characteristics, or vice versa. Pure cultures of Saaz and Frohberg top yeast, *Saccharomyces ellipsoideus II*, as well as other varieties, were found by Bau* to have no action upon melibiose even after 1 to $1\frac{1}{2}$ years; this property has been proposed by Bau as a means of distinguishing between top and bottom yeasts; the dangers of contamination and transition nullify somewhat the value of this test.

A large number of wild yeasts, mycoderms, moulds and other fungi ferment melibiose; in all such cases the presence of a special enzyme *melibiase* is assumed. Melibiase, as prepared by Bau from bottom yeast, can be heated to 110° C. (after drying) without destruction; in solution, however, its activity is destroyed at 70° C. The optimum temperature for its action is about 50° C.

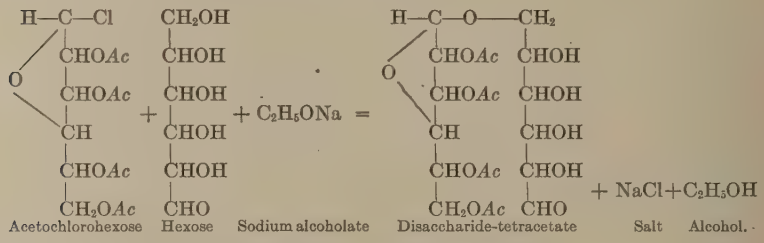
**Tests.** — Reliable qualitative tests for melibiose in presence of other sugars are lacking. The best method of procedure in case of mixtures is to remove fructose, glucose and other sugars so far as possible by a pure culture of top yeast. The melibiose may then be precipitated as its phenylosazone; the latter after recrystallization from hot water consists of fine yellow needles melting at 178° to 179° C. Melibiose-

* Chem. Ztg., **19,** 1874; **21,** 185.

phenylosazone is decomposed by benzaldehyde into melibiosone, which is hydrolyzed by emulsin into d-galactose and d-glucosone. Oxidation of melibiose or its osone with nitric acid yields mucic acid, in the same manner as with lactose.

**Synthesis.** — By allowing acetochloro-d-galactose to react with d-glucose in alcoholic solution in presence of sodium alcoholate Fischer and Armstrong* obtained a disaccharide which reduced Fehling's solution, formed osazones similar to those of melibiose and gave other reactions resembling this sugar. The sugar could not be separated in the crystalline form, but from the agreement in chemical reactions and in behavior towards yeasts and enzymes it is probably identical with melibiose.

The above synthesis is of importance as it is apparently the first to be accomplished by purely chemical means for a natural disaccharide; the method is the same as that employed for other synthetic disaccharides, and may be represented as follows:




After completion of the reaction the mixture is treated in the cold with an excess of sodium hydroxide which removes the acetyl groups with liberation of the pure disaccharide. In the above equation the terminal OH group of the hexose is made to take part in the reaction; it is evident, however, that some other OH group may enter into combination with the acetochlorohexose. Melibiose and lactose are both d-gluco-d-galactosides, each consisting of a combination of d-glucose and d-galactose with a functional aldehyde group in the glucose part of the molecule. The difference between lactose and melibiose is no doubt due to a difference in point of attachment between the OH groups of glucose and the galactoside half of the sugar. Until some method is found for determining this point of attachment the structural formula of melibiose as of all other complex sugars must remain an uncertainty.

* Ber., **35**, 3144.

**Turanose.** — $C_{12}H_{22}O_{11}$.

This disaccharide has not been found free in nature. The sugar was obtained by Alekhine * by a carefully controlled hydrolysis of the trisaccharide melezitose (p. 740).

$$\underset{\text{Melezitose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Turanose.}}{C_{12}H_{22}O_{11}}.$$

*Preparation and Properties.* — About 5.5 gms. of anhydrous melezitose are carefully warmed with 100 c.c. of 1 per cent sulphuric acid upon the water bath until the specific rotation of the sugar has fallen to $[\alpha]_D = +63°$. The solution is then neutralized with barium carbonate, and the filtrate treated with hot alcohol until turbidity develops. After cooling the precipitated sugar is purified by extracting with boiling absolute alcohol.

Turanose is obtained by the above method as a white amorphous mass, having the formula $C_{12}H_{22}O_{11}$; it is easily soluble in water and methyl alcohol but not in absolute alcohol. $[\alpha]_D = +65$ to $+68$ ($c = 30$ to 35). It is not fermentable to any great extent and reduces Fehling's solution about one-half as strong as d-glucose.

Turanose upon prolonged heating with mineral acids is hydrolyzed according to Alekhine into 2 molecules of d-glucose.

Tanret † has recently prepared turanose by hydrolyzing melezitose with 20 per cent acetic acid for 2 hours on the water bath. The acetic acid was removed by shaking out with ether and the d-glucose by fermenting with yeast. The solution was evaporated to a sirup, purified from glycerol and fatty acid fermentation products by shaking out with ether, and then extracted with boiling absolute alcohol. The filtrate on concentration and cooling yielded crystals of turanose which contained one-half molecule alcohol of crystallization and melted at 60° to 66° C. Upon drying at 100° C. anhydrous turanose was obtained of the formula $C_{12}H_{22}O_{11}$ and $[\alpha]_D = +71.8$. The reducing power was 60 per cent that of d-glucose.

According to Tanret turanose is hydrolyzed into one molecule each of d-glucose and d-fructose, turanose thus being a true isomer of sucrose. This observation cannot be reconciled with the rotation +51, obtained by Alekhine after hydrolyzing melezitose; additional investigations are needed before a final decision can be reached.

Turanose upon heating with a solution of phenylhydrazine forms a phenylosazone of the formula $C_{24}H_{32}N_4O_9$, consisting of fine yellow needles, melting at 215° to 220° C. upon rapid heating and soluble in

* Ber., **22,** 759; Ann. chim. phys. [6], **18,** 532.

† Bull. soc. chim. [3], **35,** 816.

5 parts of hot water, which solution upon cooling sets to a jelly-like mass of fine crystals.

**Gentiobiose.** — $C_{12}H_{22}O_{11}$.

This disaccharide has not been found free in nature. In the combined form it exists in the trisaccharide gentianose from which it has been obtained by Bourquelot and Herissey* by partial hydrolysis using invertase or very dilute sulphuric acid.

$$\underset{\text{Gentianose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{Gentiobiose.}}{C_{12}H_{22}O_{11}}.$$

*Preparation and Properties.*—Ten grams of gentianose are warmed upon the water bath with 100 c.c. of 0.2 per cent sulphuric acid for 30 minutes. The solution is cooled, neutralized with calcium carbonate, filtered and evaporated in vacuum to dryness. The residue is worked up with absolute alcohol and then with 95 per cent alcohol until all fructose is removed; the crude gentiobiose is purified by crystallization.

Gentiobiose, when crystallized from hot alcohol, is obtained in water-free crystals which, after drying at 115° C., melt at 190° to 195° C. The sugar is dextrorotatory and shows the phenomenon of less-rotation. $[\alpha]_D^{20} = +9.61$ (after solution). The sugar is not fermented by top yeast and this property can be utilized for separation of gentiobiose from the hydrolytic products of gentianose. While gentiobiose is not hydrolyzed by invertase, it is easily split up by emulsin into 2 molecules of d-glucose. Hydrolysis is also effected by heating with 3 per cent sulphuric acid.

$$\underset{\text{Gentiobiose}}{C_{12}H_{22}O_{11}} + H_2O = 2\,\underset{\text{d-Glucose.}}{C_6H_{12}O_6}.$$

Gentiobiose reduces Fehling's solution to about the same degree as maltose. Upon heating with phenylhydrazine an osazone is formed of melting point 142° C. These reactions show that gentiobiose contains an aldehyde group in the free reactive condition.

**Cellose.** — Cellobiose. $C_{12}H_{22}O_{11}$.

This disaccharide does not exist, so far as known, free in nature. It is apparently formed, with other intermediary carbohydrates, in the hydrolysis of cellulose to glucose by means of sulphuric acid, cellose thus bearing the same relation to cellulose as maltose bears to starch.

*Preparation.* — For the preparation† of cellose it is best to start from cellose-octacetate, which is obtained by treatment of cellulose with acetic anhydride:—7.5 gms. of finely cut filter paper are thoroughly shaken in a 200-c.c. flask with 20 c.c. of acetic anhydride; after cooling

* Compt. rend., **132,** 571; **135,** 290, 399. † Skraup, Ber., **32,** 2413.

to 70° C. the mass is treated under constant shaking with a mixture of 7 c.c. acetic anhydride and 4 c.c. concentrated sulphuric acid warmed to 70° C., and the yellowish brown solution poured into 500 to 700 c.c. of water. The amorphous yellowish colored precipitate is filtered off on linen, washed with water, pressed and recrystallized several times from 95 per cent alcohol. The cellose-octacetate thus prepared consists of white needles, melting at 228° C., and having a composition and molecular weight corresponding to the formula $C_{12}H_{14}(C_2H_3O)_8O_{11}$. The compound is soluble in hot 95 per cent alcohol, but insoluble in hot absolute alcohol, chloroform or benzol. The yield of cellose-octacetate from cellulose by this method is 26 to 27 per cent.

To prepare cellose the finely pulverized octacetate is moistened with alcohol and then treated with successive portions of a 15 per cent solution of potassium hydroxide in strong alcohol, using 5 c.c. of alcoholic potassium hydroxide for each gram of cellose-octacetate. By this treatment the octacetate is saponified and the cellose liberated.

$$\underset{\text{Cellose-octacetate}}{C_{12}H_{14}(C_2H_3O)_8O_{11}} + 8\,KOH = \underset{\text{Cellose}}{C_{12}H_{22}O_{11}} + \underset{\text{Potassium acetate.}}{8\,CH_3COOK.}$$

After 2 hours' standing the crude cellose, in the form of a granular powder, is filtered off, washed with absolute alcohol, dissolved in a little water and any free potassium hydroxide exactly neutralized with acetic acid. The solution is then filtered, evaporated to a thin sirup, 1 part absolute alcohol added and then ether to the point of turbidity. After standing several hours in the cold the precipitate is filtered off, dissolved in a little hot water and then absolute alcohol added to the appearance of turbidity. The solution is then set aside in the cold when the cellose will separate after long standing in the form of small microscopic crystals.

*Properties.* — Cellose as prepared by the above method consists of a fine white crystalline compound, which, after drying at 100° C. in a vacuum, has a composition agreeing with the formula $C_{12}H_{22}O_{11}$. The sugar melts at 225° C. under decomposition. It has a mild sweet taste, is soluble in 8 parts of cold and 1.5 parts of hot water, but insoluble in alcohol and ether. Cellose is dextrorotatory and shows the phenomenon of less-rotation, $[\alpha]_D^{20} = +26.1$ (10 minutes after solution) and + 33.7 (constant). Upon heating on the water bath with 5 per cent sulphuric acid for 7 hours cellose is hydrolyzed into 2 molecules of d-glucose.

$$\underset{\text{Cellose}}{C_{12}H_{22}O_{11}} + H_2O = 2\,\underset{\text{d-Glucose.}}{C_6H_{12}O_6}.$$

Hydrolysis is also effected by means of emulsin.

Cellose is not fermented by means of yeast. The sugar reduces Fehling's solution somewhat stronger than maltose. Upon heating with 2 parts water, 3 parts phenylhydrazine and 2 parts glacial acetic acid for 1 hour in a water bath and then adding water, cellose forms an osazone which crystallizes in the form of yellow needles melting at 198° C. These reactions show that cellose contains an aldehyde group in the free reactive condition.

**Glucosido-galactose.** — $C_{12}H_{22}O_{11}$.

This synthetic disaccharide was prepared by Fischer and Armstrong* from acetochloro-d-glucose and d-galactose (in alcoholic solution with sodium) following the same method described under the synthesis of melibiose.

The sugar was obtained only in the sirupy form; it was fermented by bottom yeast, but not by top yeast, and was hydrolyzed by emulsin. It reduced Fehling's solution.

Phenylhydrazine gave an osazone $C_{24}H_{32}N_4O_9$ consisting of yellow needles, melting at 172° to 174° C., and soluble in 120 parts of hot water.

**Galactosido-galactose.** — $C_{12}H_{22}O_{11}$.

This synthetic disaccharide was prepared by Fischer and Armstrong* from acetochloro-d-galactose and d-galactose (in alcoholic solution with sodium) following their general method as described under melibiose.

The sugar was obtained only in the sirupy condition; it was fermented neither by top nor bottom yeast but was hydrolyzed by emulsin; it reduced Fehling's solution.

Phenylhydrazine gave an osazone $C_{24}H_{32}N_4O_9$ consisting of yellow needles, melting at 173° to 175° C., and soluble in 110 parts of boiling water.

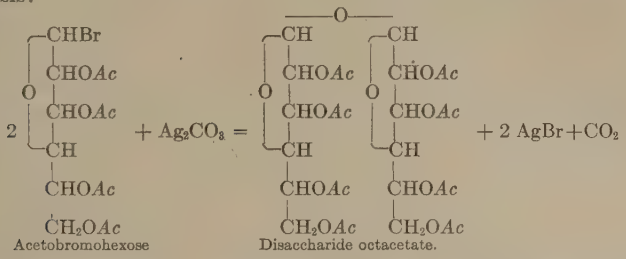
**Isotrehalose.** — $C_{12}H_{22}O_{11}$.

This disaccharide was prepared synthetically by Fischer and Delbrück† by a somewhat different process than that of Fischer and Armstrong. $\beta$-Acetobromoglucose is allowed to react in dry ethereal solution with silver carbonate, traces of water being added at intervals to promote the reaction. In this manner two molecules of acetoglucose are united by the elimination of bromine to form the octacetate of a

* Ber., **35**, 3144.

† Ber., **42**, 2776.

disaccharide. The following equation illustrates the principle of the synthesis:




The octacetate upon treatment with cold barium hydroxide solution yields barium acetate and the free disaccharide.

Isotrehalose as obtained by this process consists of a white amorphous substance, without action upon Fehling's solution and levorotatory ($[\alpha]_D = -39.4$). The absence of a free aldehyde group is a necessary consequence of this method of synthesis and the non-reducing properties of isotrehalose are thus explained. Since the two C atoms united by the O linkage are asymmetric, three stereoisomeric configurations are possible for isotrehalose; which configuration belongs to isotrehalose is uncertain.

The disaccharide upon boiling with dilute mineral acids is hydrolyzed into d-glucose.

**Dihexose Saccharides of Uncertain Nature and Constitution.** — In addition to the dihexose saccharides previously described a number of other sugars with the apparent formula $C_{12}H_{22}O_{11}$ have been reported by various investigators. Owing to the lack of confirmatory evidence brief mention is made of only a few of these compounds.

*Astragalose* reported by Frankforter* in the poisonous fruit of *Astragalus caryocarpus*. It reduces Fehling's solution and forms a phenylhydrazone melting at 186° to 188° C.

*Parasaccharose* formed according to Jodin† by the action of a variety of *Torula* upon sucrose solutions in presence of ammonium phosphate. It forms fine crystals, is dextrorotatory ($[\alpha]_j = +108$), reduces Fehling's solution and is hydrolyzed by heating with acids.

*Pharbitose* reported by Kromer‡ in the seeds of *Pharbitis Nil*. $[\alpha]_D = +109.53$.

*Pseudostrophanthobiose* formed according to Feist§ in the hydrolysis of pseudostrophanthin a glucoside occurring in *Strophanthus hispidus*.

* Chem. Centralbl. (1900) II, 484.
† Compt. rend., **53**, 1252.
‡ Archiv. Pharm., **234**, 459.
§ Ber., **33**, 2063, 2069.

*Racefoliobiose* reported by Boettinger* in grape leaves.

*Revertose* (Revertobiose) formed according to Hill† by the action of maltase or takadiastase upon concentrated glucose solutions (see p. 704)

*Amygdalinbiose* liberated according to Giaju‡ by the action of the juice of *Helix pomatia* (the so-called "edible snail") upon amygdalin. It is non-reducing and gives only d-glucose upon hydrolysis.

### Hexose-heptose Saccharides

$$O\begin{matrix}\nearrow C_6H_{11}O_5 \\ \searrow C_7H_{13}O_6\end{matrix}$$

**Galactosido-glucoheptose.** — $C_{13}H_{24}O_{12}$.

This synthetic disaccharide was obtained by Fischer§ through reduction of the lactone of lactose carboxylic acid (p. 717) by means of sodium amalgam. The sugar, which has not been isolated in the crystalline form, is hydrolyzed by mineral acids into d-galactose and d-glucoheptose.

$$\underset{\text{Galactosido-glucoheptose}}{C_{13}H_{24}O_{12}} + H_2O = \underset{\text{d-Galactose}}{C_6H_{12}O_6} + \underset{\text{d-Glucoheptose.}}{C_7H_{14}O_7}.$$

**Glucosido-glucoheptose.** — $C_{13}H_{24}O_{12}$.

This sugar was prepared by Fischer‖ through reduction of the lactone of maltose carboxylic acid (p. 703) by sodium amalgam. The sugar, which has not been obtained in the crystalline form, gives upon hydrolysis d-glucose and d-glucoheptose.

* Chem. Ztg., **25,** 24.
† Chem. News, **83,** 578.
‡ Chem. Ztg., **34,** 430.
§ Ber., **23,** 937; Reinbrecht, Ann., **272,** 197.
‖ Ber., **23,** 937; Reinbrecht, Ann., **272,** 197.

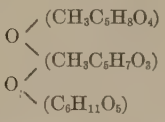
# CHAPTER XXI

## THE TRISACCHARIDES AND TETRASACCHARIDES

### Trisaccharides

#### Methylpentose-hexose Saccharides

**RHAMNINOSE.**




**Occurrence and Preparation.** — Rhamninose is formed* by the hydrolysis of the glucoside xanthorhamnin by means of the enzyme *rhamninase,* which occurs associated with xanthorhamnin in Persian berries (the fruit of *Rhamnus infectoria*).

Rhamninase is prepared by extracting Persian berries with water; the enzyme is precipitated from the extract by means of alcohol. To obtain rhamninose xanthorhamnin is treated in aqueous solution between 45° and 70° C. with a solution of rhamninase. The solution is then shaken out with ether to remove any unchanged xanthorhamnin, and then after clarification by means of bone black evaporated to a sirup; the sirup is extracted with hot alcohol, the alcohol solution evaporated to a sirup, and set aside for crystallization. The sugar is purified by recrystallizing.

**Properties and Reactions.** — Rhamninose as above prepared consists of white crystals with a composition and molecular weight corresponding to the formula $C_{18}H_{32}O_{14}$. The sugar has a mild, sweet taste, shows incipient fusion at 135° to 140° C. with decomposition and is soluble in water and hot alcohol, but not in ether. Rhamninose is levorotatory ($[\alpha]_D = -41°$) and reduces Fehling's solution.

Upon treatment with sodium amalgam rhamninose is reduced to the alcohol rhamninite $C_{18}H_{34}O_{14}$ ($[\alpha]_D = -57°$), which upon heating with dilute acids is hydrolyzed as follows:

$$\underset{\text{Rhamninite}}{C_{18}H_{34}O_{14}} + 2\,H_2O = \underset{\text{Dulcite}}{C_6H_{14}O_6} + 2\,\underset{\text{Rhamnose.}}{C_6H_{12}O_5}.$$

Upon treatment with bromine in aqueous solution rhamninose is

* Tanret, Compt. rend., **129,** 725.

oxidized to rhamninotrionic acid $C_{18}H_{32}O_{15}([\alpha]_D = -94.3$ for acid-lactone mixture), which upon heating with dilute acids is hydrolyzed as follows:

$$\underset{\text{Rhamnino-trionic acid}}{C_{18}H_{32}O_{15}} + 2\,H_2O = \underset{\text{d-Galactonic acid}}{C_6H_{12}O_7} + 2\,\underset{\text{Rhamnose.}}{C_6H_{12}O_5}.$$

Upon oxidation with nitric acid rhamninose yields mucic acid.

Upon warming with dilute hydrochloric or sulphuric acid rhamninose is hydrolyzed as follows:

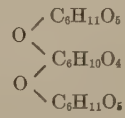
$$\underset{\text{Rhamninose}}{C_{18}H_{32}O_{14}} + 2\,H_2O = \underset{\text{d-Galactose}}{C_6H_{12}O_6} + 2\,\underset{\text{Rhamnose.}}{C_6H_{12}O_5}.$$

These various reactions show that rhamninose is composed of 2 rhamnose and 1 d-galactose radicals, the latter having its aldehyde group in a free reactive condition.

Rhamninose is not fermented by yeast. Invertase, diastase and emulsin have no hydrolytic action.

Rhamninose, through the presence of a reactive aldehyde group, forms a phenylhydrazone and osazone, but these compounds owing to their extreme solubility have not been obtained in a pure condition.

## Trihexose Saccharides




**Raffinose.** — Melitriose. Gossypose.

$$C_{18}H_{32}O_{16} + 5\,H_2O.$$

**Occurrence.** — Raffinose is the best known and most widely distributed of the trisaccharides. The name raffinose was first given to a new sugar discovered by Loiseau* in 1876 in the impure molasses obtained from refining beet sugar (French, *raffiner* = to refine). The same sugar had been previously isolated, however, by Johnston† from Eucalyptus manna in 1843, afterwards described by Berthelot‡ as melitose. Tollens,§ however, showed that melitose was identical with Loiseau's raffinose and also proved the same to be true of gossypose, a sugar found by Ritthausen‖ and by Böhm¶ in cottonseed meal. The identity, thus established by Tollens, was important for it opened the way to investigations which established the wide occurrence of raffinose in the vegetable kingdom. In addition to the sources just mentioned raffinose has been found in barley and other grains, in young

* J. fabr. sucre., **24,** 52; **26,** 22.
† J. prakt. Chem. [1], **29,** 485.
‡ Ann. chim. phys. [3], **46,** 66.
§ Ber., **18,** 26.
‖ J. prakt. Chem. [2], **29,** 351.
¶ J. prakt. Chem. [2], **30,** 37.

wheat sprouts (up to 6.9 per cent of the dry substance) and in many other plant substances.

Raffinose has attracted most attention from its occurrence in sugar-beet products. It had been the opinion of many chemists that raffinose was formed during the process of manufacture by the action of alkalies upon the sucrose, invert-sugar and other constituents of the juice; Lippmann,* however, was able to separate raffinose directly from expressed beet juice, thus proving that the sugar was formed during the growth of the beet. The amount ordinarily occurring in sugar beets is only from 0.01 per cent to 0.02 per cent; under certain conditions, however, the percentage of raffinose may greatly exceed this, the result, perhaps, of abnormal climatic causes, such as drought, excessive rain, freezing, etc., the exact rôle of these various factors being as yet not clearly understood. The synthesis of raffinose in the plant is apparently connected with a saccharification of galactan substances (pectin, etc.) in presence of sucrose, the result no doubt of enzyme action.

Raffinose has also been reported by Pellet† and other investigators in sugar-cane molasses, although the claims for this have been disputed by Lippmann.‡ Until additional evidence is obtained the occurrence of raffinose in sugar-cane products must be regarded as exceedingly unusual.

**Preparation of Raffinose.** — Raffinose may be prepared from Eucalyptus manna (a secretion from certain Eucalyptus trees, as *E. viminalis* and *E. Gunnii*) by extracting the manna with hot water, clarifying the extract with bone black and evaporating to the point of crystallization. A more common material for preparing raffinose is cottonseed meal; the method of Zitkowski§ is as follows:

*Preparation of Raffinose from Cottonseed Meal.* — Cotton-seed meal is extracted with cold water for 1 hour and the filtered extract made faintly alkaline with milk of lime. The filtrate from precipitated matter is polarized (in terms of sucrose) and then treated at low temperature with finely powdered quick lime in the proportion of 125 parts CaO to 100 parts of sugar. The precipitate of calcium raffinosate is filtered off, washed with cold lime water and then, after dissolving in hot water, carbonated at 80° C. almost to neutrality. After filtering from calcium carbonate the solution is concentrated to a sirup of about 75 degrees Brix and set aside in the cold to crystallize. Priming with a crystal of raffinose will hasten the process. The crystals of raffinose are filtered off,

* Ber., **18**, 3087.
† Bull. assoc. chim. sucr. dist., **14**, 139.
‡ Deut. Zuckerind., **22**, 1439.
§ Am. Sugar Ind., **12**, 324.

washed with 90 per cent alcohol and purified by recrystallization. In one experiment by this method 600 gms. of raffinose hydrate were obtained from 150 lbs. of cottonseed meal.

*Preparation of Raffinose from Beet Molasses.* — A number of processes have been devised for preparing raffinose from beet molasses. Scheibler* first noted that absolute methyl alcohol had a high solvent action upon raffinose (9.8 gms. raffinose anhydride in 100 c.c.) and a low solvent action upon sucrose (0.4 gm. sucrose in 100 c.c.). Using this observation as a basis Burkhard† employed the following method: A low-grade beet molasses rich in raffinose (preferably from the strontium monosaccharate process) is absorbed upon clean dry wood shavings and, after thoroughly drying in a vacuum, extracted with absolute methyl alcohol. The extract is diluted with water, the alcohol evaporated and the solution boiled, during addition of crystallized strontium hydrate with constant stirring, until a permanent crust of crystals begins to form upon the surface. The strontium compound is filtered off, washed with hot saturated strontium hydroxide solution and then carbonated in suspension with water. The solution is evaporated, the sirup dissolved at 60° to 70° C. in the exactly necessary amount of 80 per cent alcohol, and then set aside for 24 to 48 hours, when raffinose will crystallize out.

The precipitation of raffinose from molasses as lead raffinosate by means of ammoniacal lead acetate, lead carbonate or litharge has also been successfully employed. Zitkowski‡ has used the following process, which is based upon the insolubility of lead raffinosate and the solubility of lead saccharate at high temperature:

Thirty pounds of the molasses are diluted to about 50 degrees Brix, brought to a boil with the addition of 3 pounds of litharge and filtered, this being done for the purpose of precipitating some of the lead salts that form. Then 3 pounds more of lead oxide are taken and just sufficient of the purified molasses filtrate added to form a thin paste. The mixture is stirred for about an hour in the cold when the formation of lead saccharate begins; the mass which becomes stiff is then allowed to set twenty-four hours. The main portion of the molasses solution is then brought to a boil and the lead saccharate added in small portions at a time in order to disintegrate the mass. When all of the lead saccharate is added, the mixture is kept at boiling for about thirty minutes, then filtered and thoroughly washed with water. The lead com-

* Ber., **19,** 2868.
† Neue Ztschr. Rübenzuckerind., **20,** 16.
‡ Am. Sugar Ind., **13,** 8.

pound thus obtained is decomposed with carbon dioxide, filtered and evaporated to a light sirup. The sirup is treated with blood black and again filtered, evaporated on a water bath to a heavy sirup and set away to crystallize. The filtration of the lead raffinosate should be performed as hot and as quickly as possible, otherwise considerable quantities of lead saccharate will be precipitated. The crystallization of the final sirup can be accelerated by priming with a pinch of pure raffinose.

**Properties.** — Raffinose crystallizes from aqueous solution in the form of long pointed needles or prisms, with a composition and molecular weight corresponding to the formula $C_{18}H_{32}O_{16} + 5\ H_2O$. The crystals upon gradual warming at 80° C. for several hours and then at 100° to 105° C. lose all their water and pass without melting into the anhydride. Upon rapid heating the crystals melt in their water of crystallization below 100° C.; under this condition the last traces of water are removed only at 125° to 130° C. when decomposition sets in with brown coloration and odor of caramel. The sensibility of raffinose to destructive changes upon rapid heating is shown by raffinose-containing beet sugar, which darkens at 120° to 125° C.; while ordinary beet sugar, free from raffinose, is not as a rule affected.

Raffinose anhydride has the formula $C_{18}H_{32}O_{16}$ and consists of a white amorphous hygroscopic mass which upon exposure to moist air reabsorbs after several days the entire amount of water of crystallization.

Raffinose hydrate is more soluble than sucrose in hot water, but less soluble in cold water; 14 to 15 parts of water are necessary to dissolve raffinose at 0° C., 9 parts at 10° C. and 6 parts at 16° C. Supersaturated solutions are easily formed from which the raffinose is deposited upon long standing. Raffinose is insoluble in absolute ethyl alcohol or in ether; its solubility in absolute methyl alcohol is considerable as previously stated. Raffinose, through its property of combining with water of hydration, seems to possess the property of throwing sucrose out of solution.*

*Influence of Raffinose Upon the Crystalline Form of Sucrose.* — The presence of raffinose exerts a peculiar effect in giving crystals of sucrose a pointed needle-like structure; 3 per cent raffinose in a sugar sirup may produce a sensible elongation of the grain, the pointed character of the crystals increasing with the amount of raffinose present. This alteration in grain is frequently noted in the crystallization of low-grade beet products and is *usually* an indication of the presence of raffinose.

* Herzfeld, Z. Ver. Deut. Zuckerind., **42**, 207.

It must be remembered, however, that other impurities (organic lime salts, caramelization products, etc.) may produce under certain conditions a pointed grain, especially when crystallization takes place from viscous supersaturated solutions. On the other hand, raw beet sugars may contain 4 to 5 per cent of raffinose without alteration of grain, in case the raffinose remains dissolved in the molasses coating of the crystals.*

*Specific Rotation.* — In aqueous solution raffinose is strongly dextrorotatory, the value of $[\alpha]_D$ for the hydrate ranging from + 104 to + 105.7, according to different observers for different preparations of sugar. For purposes of analysis the value + 104.5 may be used without serious error for raffinose hydrate, and + 123.2 for the anhydride $\left(\frac{104.5}{84.84} \times 100\right)$. The observations of Creydt† show a slight falling off in specific rotation with increase in temperature.

**Reactions.** — Raffinose does not reduce Fehling's solution, behaving in this respect similar to sucrose. Raffinose also shows the same resistance to the action of alkalies as sucrose. Both of these reactions indicate the absence in raffinose of a functional aldehyde or ketone group. Upon oxidation with nitric acid raffinose yields a mixture of acids, of which oxalic, saccharic and mucic are the most important. The yield of mucic acid from raffinose hydrate by the method of Tollens is 22 to 23 per cent, which corresponds to about 30 per cent galactose (yield of mucic acid from galactose by same method is 77 to 78 per cent).

*Hydrolysis of Raffinose by means of Acids.* — Upon heating with dilute hydrochloric or sulphuric acid raffinose is hydrolyzed according to the following equation:

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + 2\,H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{d-Galactose}}{C_6H_{12}O_6} + \underset{\text{d-Fructose.}}{C_6H_{12}O_6}.$$

The total yield of reducing sugars according to this equation would be 107.1 per cent for the anhydride and 90.9 per cent for the hydrate. The yield of galactose from raffinose hydrate according to theory would be 30.3 per cent, which agrees closely with the value calculated from the yield of mucic acid.

The hydrolysis of raffinose, as Tollens‡ first showed, proceeds in several phases. The first step in the reaction is the splitting off of fructose; glucose and galactose appear only at a later stage of the reaction. Scheibler and Mittelmeier§ showed that by moderate warming with

* Herzfeld, Z. Ver. Deut. Zuckerind, **39,** 661.
† Z. Ver. Deut. Zuckerind. **37,** 153.
‡ Ann., **232,** 169.
§ Ber., **22,** 1678, **26,** 2930.

dilute acid (as 10 gms. raffinose + 90 c.c. water + 6 c.c. hydrochloric acid 1.19 sp. gr. 10 minutes at 68°) the reaction proceeds as follows:

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}}.$$

It is only by prolonged heating with more concentrated acid that the melibiose (see p. 723) is hydrolyzed into d-glucose and d-galactose, the complete conversion of the melibiose being accompanied by a partial destruction of the fructose. As a rule less than 90 per cent of the theoretical yield of monosaccharides is obtained by the acid hydrolysis of raffinose under the most favorable conditions.

During the hydrolysis of raffinose the specific rotation undergoes a marked decrease, the final reading depending upon the extent of the hydrolysis. For the ordinary method of Clerget inversion the specific rotation of raffinose hydrate decreases from + 104.5 to about + 53 or + 54, which corresponds to the mixture of fructose and melibiose required by the preceding equation (30.30 per cent fructose and 57.57 per cent melibiose).* Upon prolonged heating with acid the specific rotation of raffinose was found by Tollens to sink as low as + 20. The theoretical value† for a mixture of 30.3 per cent each of d-glucose, d-galactose and d-fructose is about + 12.50; decomposition of fructose, however, sets in before this limit is reached so that higher figures of variable value are obtained.

*Hydrolysis of Raffinose by Means of Enzymes.* — The hydrolysis of raffinose can also be effected by means of enzymes, the nature of the reaction depending upon the character of the enzyme.

Invertase hydrolyzes raffinose into d-fructose and melibiose, as already described under the latter sugar. Emulsin, on the other hand, hydrolyzes raffinose into d-galactose and sucrose. The general formula for both of these reactions is the same:

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \begin{cases} + \text{ invertase} = \text{d-fructose} + \text{melibiose.} \\ + \text{ emulsin} = \text{d-galactose} + \text{sucrose.} \end{cases}$$

For the complete hydrolysis of raffinose into its component monosaccharides the action of two different enzymes is necessary and it is

* The 57.57 per cent melibiose anhydride would give a rotation of $0.5757 \times +143 = +82.3$; the 30.30 per cent fructose would give a rotation of $0.303 \times -92 = -27.9$. The combination of those effects would be $+82.3 - 27.9 = +54.4$.

† The 30.3 per cent d-glucose would give a rotation of $0.303 \times +52.5 = +15.9$; the 30.3 per cent d-galactose would give $0.303 \times +81 = +24.5$; the 30.3 per cent d-fructose would give $0.303 \times -92 = -27.9$. The combination of these effects would be $+15.9 + 24.5 - 27.9 = + 12.5$.

evident that this can be accomplished by the action of invertase and melibiase (p. 723), or by that of emulsin followed by invertase.

These reactions may be explained by assuming the following arrangement for the monosaccharide groups in raffinose.

$$\underset{\text{d-Fructose radical}}{C_6H_{11}O_5} - \overset{*}{O} - \underset{\text{d-Glucose radical}}{C_6H_{10}O_4} - \overset{\dagger}{O} - \underset{\text{d-Galactose radical}}{C_6H_{11}O_5}.$$

Sucrose — Melibiose.

The hydrolysis by means of invertase or weak acids takes place at the O atom marked *; the hydrolysis by means of emulsin takes place at the O atom marked †.

**Fermentation of Raffinose.** — The fermentation of raffinose by means of yeast depends upon the character of the enzymes which are present. Bottom yeasts, which contain both invertase and melibiase and can thus effect a complete hydrolysis, ferment raffinose completely, although somewhat more slowly than sucrose. Top yeasts, on the other hand, which do not ordinarily contain melibiase, ferment only the fructose part of the molecule with a corresponding reduction in the yield of alcohol. The theoretical equations for the two fermentations would be:

Bottom yeast, $\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + 2\,H_2O = \underset{\text{Alcohol (54.78 per cent)}}{6\,C_2H_5OH} + \underset{\text{Carbon dioxide.}}{6\,CO_2}.$

Top yeast, $\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Alcohol (18.26 per cent)}}{2\,C_2H_5OH} + \underset{\text{Carbon dioxide}}{2\,CO_2} + \underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}}.$

The yield of alcohol, expressed in percentage of raffinose anhydride, is somewhat less in actual practice than indicated above.

A number of moulds (species of *Monilia*, *Amylomyces* and *Aspergillus*) also hydrolyze and ferment raffinose. Other moulds, as *Aspergillus niger* and *Penicillium glaucum*, hydrolyze raffinose but instead of producing alcohol form acid oxidation products such as oxalic and succinic acids.

Special bacteria also ferment raffinose with production of lactic and butyric acids.

**Compounds of Raffinose.** — Owing to the absence of a reactive aldehyde or ketone group, raffinose does not form hydrazones, osazones, mercaptals, ureides, oximes or any other of the numerous compounds which are characteristic of reducing sugars.

Upon heating raffinose with acetic anhydride Scheibler and Mittelmeier* obtained a hendecacetate, $C_{18}H_{21}(C_2H_3O)_{11}O_{16}$. After recrys-

* Ber., **23**, 1438.

tallizing from hot absolute alcohol, the compound was obtained as white leaflets melting at 99° to 101° C.; it is soluble in absolute alcohol, aniline, chloroform and benzol and is dextrorotatory, $[\alpha]_D = +92.2$. Tanret* has prepared a dodecacetate of raffinose, $C_{18}H_{20}(C_2H_3O)_{12}O_{16}$; $[\alpha]_D = +100.3$.

Stolle† obtained by the usual methods a raffinose octobenzoate, $C_{18}H_{24}(C_7H_5O)_8O_{16}$; the compound consists of a white powder, melting at 98° C. and showing in glacial acetic acid $[\alpha]_D = +4.1$.

Raffinose forms a number of compounds with the alkalies, alkaline earths and other metals. These compounds have been especially studied by Beythien and Tollens‡ from whose work the following examples are taken.

*Sodium raffinosate*, $C_{18}H_{31}NaO_{16}$, is obtained by precipitating an alcoholic raffinose solution with a one-molecular proportion of sodium alcoholate. By taking a two-molecular proportion of sodium alcoholate the compound $C_{18}H_{31}NaO_{16} + NaOH$ is obtained. Both substances are white amorphous powders.

*Barium raffinosates*, corresponding to the formulæ $C_{18}H_{32}O_{16}{\cdot}BaO$ and $C_{18}H_{32}O_{16}{\cdot}2BaO$, are obtained by mixing barium hydroxide and raffinose solutions in presence of alcohol in the proper molecular proportions. The compounds were obtained as white amorphous substances of imperfect purity.

*Strontium raffinosate*, of the formula $C_{18}H_{32}O_{16}{\cdot}2\,SrO + H_2O$, is obtained by heating a solution of strontium hydroxide and raffinose, as a sticky mass which becomes granular upon long boiling or upon addition of alcohol. The compound consists of a white granular amorphous powder which loses its water of combination at 80° C. Raffinose compounds containing 1 SrO and 3 SrO have not as yet been obtained.

*Calcium raffinosate*, $C_{18}H_{32}O_{16}{\cdot}3\,CaO + 3\,H_2O$, is obtained by heating a raffinose solution saturated with calcium hydroxide. The compound consists of a white amorphous powder which loses its water of combination at 100° C.

Lindet§ by dissolving calcium hydroxide in a cold raffinose solution obtained the compound $C_{18}H_{32}O_{16}{\cdot}2\,CaO + 5\,H_2O$. Lindet also noted upon treating a solution of sucrose, raffinose and lime with alcohol that calcium raffinosate was dissolved mostly by weak and calcium saccharate mostly by strong alcohol. This method has been proposed as a means of separating sucrose and raffinose, but is inferior to the methods described under preparation of raffinose.

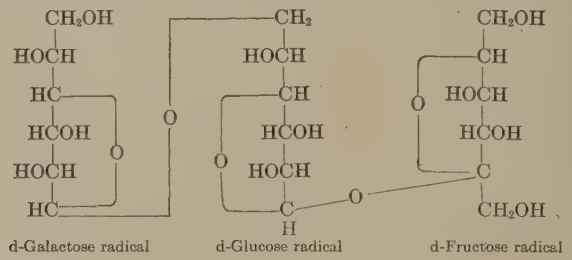
* Bull. soc. chim. [3], **13**, 261.
† Z. Ver. Deut. Zuckerind., **51**, 33.
‡ Z. Ver. Deut. Zuckerind., **39**, 894.
§ J. fabr. sucre, **31**, 19.

*Lead raffinosate*, $C_{18}H_{32}O_{16}\cdot 3\,PbO$, was obtained by Lippmann* upon treating raffinose solutions with ammoniacal lead subacetate. Lead raffinosate can also be prepared by heating raffinose solutions with litharge or, according to Wohl,† more advantageously by heating with lead saccharate (see under preparation of raffinose).

**Tests for Raffinose.** — As in the case of most other sugars the only absolute test for raffinose is the separation of the sugar in pure crystalline form and the determination of its specific rotation, products of hydrolysis and other properties. For the separation of raffinose any of the methods described under preparation may be used.

It is evident from its composition that raffinose after hydrolysis will give any of the reactions described for d-glucose, d-fructose and d-galactose, so that ordinary qualitative tests are valueless when several of these sugars are present. The removal of fermentable sugars by a pure culture of top yeast, and examination of the residual sugars for melebiose may be used for corroboration. For quantitative methods of determining raffinose see page 281.

**Configuration.** — The probable arrangement of the monosaccharide groups in raffinose has already been given; the manner in which these different groups are combined has not, however, been established. The following configuration is regarded at present as the one which corresponds most closely to the properties of raffinose.




The synthesis of raffinose has not as yet been effected.

**Melezitose.** — Melezitriose. $C_{18}H_{32}O_{16} + 2\,H_2O$.

**Occurrence.** — This trisaccharide, first observed in 1833 by Bonastre,‡ has been found for the most part as a constituent of the secretions of different trees, such as manna of *Pinus larix*, manna of *Alhagi Maurorum* (Turkestan manna), Lahore manna, honey dew of the

* Z. Ver. Deut. Zuckerind., **35**, 257.
† Deut. Zuckerind., **25**, 1125.
‡ J. pharm. chim. [2], **8**, 335; **19**, 443, 626.

linden, etc. The sugar was named melezitose by Berthelot* in 1856, and was supposed by him to be a disaccharide; Alekhine,† however, proved the sugar to be without question a trisaccharide.

**Preparation.** — For the preparation‡ of melezitose Turkestan manna§ (Turandjabine) is extracted with warm water, and the filtered solution concentrated to a sirup; an excess of methyl alcohol is then added when crystallization takes place within 24 hours. The crude sugar is purified by means of bone black; coloring matter is precipitated by a little barium hydroxide solution, any excess of the latter being removed with ammonium carbonate. The filtrate is again concentrated and crystallized in presence of methyl alcohol. The yield of pure melezitose by this method is 36 per cent of the manna taken.

**Properties.** — Melezitose as ordinarily prepared consists of white rhombic crystals with a composition and molecular weight corresponding to the formula $C_{18}H_{32}O_{16} + 2\,H_2O$. The crystals of the hydrate effloresce upon exposure to the air and with gradual elevation of temperature give up their water, passing without decomposition into the anhydride $C_{18}H_{32}O_{16}$. The latter may also be obtained directly upon crystallizing melezitose from hot concentrated aqueous or alcoholic solutions. Melezitose anhydride consists of a white crystalline powder which upon rapid heating melts at 148° to 150° C.; it is soluble in 2.73 parts of water at 17.5° C. and 0.32 part at 100° C., it is slightly soluble in hot alcohol but insoluble in ether.

Melezitose is dextrorotatory, $[\alpha]_D$ for the anhydride $= +\,88.5$ and for the hydrate $+\,83$. Mutarotation does not exist.

**Reactions and Hydrolysis.** — Hot solutions of dilute alkalies are without action upon melezitose. The sugar like raffinose does not reduce Fehling's solution. Upon heating with dilute hydrochloric or sulphuric acid melezitose is hydrolyzed, the reaction proceeding as Alekhine‖ found in two distinct stages.

The first phase of the hydrolysis consists in the conversion of melezitose into d-glucose and the disaccharide turanose.

$$\underset{\text{Melezitose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + \underset{\text{Turanose.}}{C_{12}H_{22}O_{11}}. \qquad (1)$$

This part of the hydrolysis is best performed by means of 20 per cent hydrochloric acid in the cold or upon warming with 1 per cent sulphuric

* Compt. rend., **47,** 224.

† Bull. soc. chim. [2], **46,** 824.

‡ Maquenne's "Les Sucres," p. 701.

§ Turkestan manna, or Turandjabine, is used in the Orient for sweetening drinks. It is sold in Tashkend under the name of Koum-tchakar (Koum = sand; tchakar = sugar).

‖ Ann. chim. phys. [6], **18,** 532.

acid; the rotation of the sugar falls from + 83 for the hydrate to about + 63 which marks the completion of the first step in the hydrolysis.

Upon prolonged boiling with dilute hydrochloric or sulphuric acid, melezitose is completely hydrolyzed into d-glucose:

$$\underset{\text{Melezitose}}{C_{18}H_{32}O_{16}} + 2\,H_2O = 3\,\underset{\text{d-Glucose.}}{C_6H_{12}O_6}. \qquad (2)$$

In this second phase of the hydrolysis the turanose, which is first formed, is split up into two molecules of d-glucose, and the specific rotation falls to about + 51 which agrees very closely with that of d-glucose.

In the second stage of the hydrolysis, according to Tanret, turanose is hydrolyzed into d-glucose and d-fructose, so that melezitose would give upon complete hydrolysis 2 molecules of d-glucose and 1 molecule of d-fructose. The calculated rotation of the latter mixture would be about + 4.5 which does not agree with the value obtained by Alekhine for hydrolyzed melezitose. Additional investigation is needed to decide the question. (See under turanose, p. 725).

**Compounds.** — Upon acetylating with acetic anhydride Alekhine obtained a hendecacetate, $C_{18}H_{21}(C_2H_3O)_{11}O_{16}$, which consists of large monoclinic prisms melting at 170° C.; it is non-reducing, insoluble in water, soluble in alcohol and benzol and shows in benzol solution $[\alpha]_D = + 110.44$.

**Fermentation.** — Melezitose is not fermented by yeast. *Aspergillus niger* effects a slow hydrolysis at 50° C. into d-glucose and turanose, but is without further change.

GENTIANOSE. — $C_{18}H_{32}O_{16}$.

**Occurrence.** — This trisaccharide was discovered by Meyer* in the roots of *Gentiana lutea* and, according to Bourquelot and Nardin,† occurs also in other members of the Gentian family.

**Preparation.** — Fresh Gentian roots are ground and extracted for 20 to 25 minutes with boiling 95 per cent alcohol using a reflux condenser. The alcoholic extract is pressed out, the alcohol distilled off, excess of calcium carbonate added to neutralize acid and the solution filtered. The filtrate is evaporated to a sirup, freed from gummy matter by precipitation with 95 per cent alcohol, and the clear alcoholic solution filtered and set aside. Crystals of gentianose separate after about 2 weeks' standing; they are filtered off and purified by recrystallization.

The Gentian roots employed for the preparation of gentianose must be fresh; old or dried roots or aqueous extracts do not yield gentianose

* Ber., **15**, 530; Z. physiol. Chem., **6**, 135. † Compt. rend., **126**, 280.

on account of its hydrolysis by an enzyme into d-fructose and gentiobiose.

**Properties.** — Gentianose is obtained in the form of white crystals, melting at 209° to 210° C. and having a composition and molecular weight corresponding to the formula $C_{18}H_{32}O_{16}$. The sugar is easily soluble in cold water, slightly soluble in boiling alcohol, insoluble in absolute alcohol and ether. Gentianose is dextrorotatory, $[\alpha]_D = +33.4$ (Meyer), + 31.25 (Bourquelot and Nardin). After boiling the solution Meyer noted in one instance $[\alpha]_D = +65.7$. Whether this is due to mutarotation or to some chemical change is uncertain. Gentianose does not reduce Fehling's solution.

**Hydrolysis of Gentianose.** — Gentianose upon heating with acids undergoes hydrolysis, the reaction proceeding as shown by Bourquelot and Herissey in two distinct stages. In the first phase of the hydrolysis gentianose is hydrolyzed into d-fructose and the disaccharide gentiobiose (p. 726).

$$\underset{\text{Gentianose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{Gentiobiose.}}{C_{12}H_{22}O_{11}}.$$

This step of the hydrolysis is best carried out as described under gentiobiose.

Upon heating gentianose with 3 per cent sulphuric acid, the gentiobiose which is first split off is hydrolyzed into 2 molecules of d-glucose (p. 726). The complete hydrolysis of gentianose is then expressed as follows:

$$\underset{\text{Gentianose}}{C_{18}H_{32}O_{16}} + 2\,H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{d-Glucose.}}{2\,C_6H_{12}O_6}.$$

**Fermentation and Action of Enzymes.** — Gentianose is only one-third fermented by yeast, the invertase of the latter splitting off d-fructose, which is fermented, and the gentiobiose remaining unfermented. *Aspergillus niger* contains enzymes, which effect the complete hydrolysis of gentianose, and thus ferment the sugar entirely. Diastase and emulsin are without action on gentianose. Emulsin, however, can hydrolyze gentiobiose, so that yeast in presence of emulsin can ferment gentianose completely. According to Bourquelot emulsin seems to be accompanied at times by an enzyme which hydrolyzes gentianose into d-glucose and sucrose.

**Configuration.** — The arrangement of the monosaccharide groups in gentianose is probably as follows:

$$\underset{\text{d-Fructose radical}}{C_6H_{11}O_5} - \overset{*}{O} - \underset{\text{d-Glucose radical}}{C_6H_{10}O_4} - \overset{\dagger}{O} - \underset{\text{d-Glucose radical}}{C_6H_{11}O_5}.$$

Sucrose — Gentiobiose

The hydrolysis by means of weak acids or invertase takes place at the O atom marked *. The hydrolysis into d-glucose and sucrose would take place at the O atom marked †. The non-reducing properties of gentianose show that none of its monosaccharide components contains a reactive aldehyde or ketone group; the manner in which the monosaccharide groups of gentianose are united is not known, so that the configuration of this trisaccharide still remains uncertain.

MANNATRISACCHARIDE. — $C_{18}H_{32}O_{16}$.

**Occurrence.** — Mannatrisaccharide was discovered by Tanret* in the manna of the ash tree (*Fraxinus Ornus, F. rotundifolia, etc.*), of which it makes up from about 6 to 16 per cent. Ash manna also contains from 40 to 60 per cent of mannite and a smaller amount of mannatetrasaccharide or stachyose (see p. 747); in the preparation of mannatrisaccharide it is necessary to remove these accompanying constituents by fractional crystallization and precipitation.

**Preparation.** — Ash manna is extracted with 70 per cent alcohol, and the mannite which crystallizes out separated by filtration. The mother liquor is then evaporated to dryness and extracted first with boiling 95 per cent and then with boiling 85 per cent alcohol. In this manner the mannite is mostly eliminated and a residue obtained showing a rotation of about $[\alpha]_D = +\ 140$. The solution of the residue is then fractionally precipitated with barium hydroxide in presence of alcohol; the two fractions are decomposed separately with carbon dioxide to precipitate barium and the solutions evaporated to crystallization. The crude sugars are recrystallized several times when mannatrisaccharide is obtained from one portion and mannatetrasaccharide from the other.

**Properties.** — Mannatrisaccharide is a white sweet crystalline substance, very hygroscopic and melting at about 150° C. It is easily soluble in water, soluble at 15° C. in 60 parts 85 per cent and in 130 parts 90 per cent alcohol and at 78° C. in 200 parts absolute alcohol. Mannatrisaccharide reduces Fehling's solution about one-third as strong as d-glucose and is strongly dextrorotatory, $[\alpha]_D = +\ 167$.

Upon heating with dilute acids mannatrisaccharide is hydrolyzed into 1 molecule of d-glucose and 2 molecules of d-galactose.

$$\underset{\text{Mannatrisaccharide}}{C_{18}H_{32}O_{16}} + 2\,H_2O = \underset{\text{d-Glucose}}{C_6H_{12}O_6} + 2\,\underset{\text{d-Galactose.}}{C_6H_{12}O_6}$$

Upon oxidation with bromine in aqueous solution mannatrisaccharide

* Compt. rend., **134**, 1586.

is oxidized to mannatrionic acid, $C_{18}H_{32}O_{17}$, which upon warming with dilute acids is hydrolyzed as follows:

$$\underset{\text{Mannatrionic acid}}{C_{18}H_{32}O_{17}} + 2\,H_2O = \underset{\text{d-Gluconic acid}}{C_6H_{12}O_7} + \underset{\text{d-Galactose.}}{2\,C_6H_{12}O_6}$$

This reaction shows that the functional aldehyde group of mannatrisaccharide belongs to the d-glucose group.

Mannatrisaccharide is slowly fermented by yeast, but the completeness of this fermentation has not been determined.

Owing to the presence of a reactive aldehyde group mannatrisaccharide forms with phenylhydrazine a yellow amorphous hydrazone, easily soluble in water and alcohol, and a crystalline osazone melting at 122° C. and quite soluble in water.

Mannatrisaccharide forms a dodecacetate, $C_{18}H_{20}(C_2H_3O)_{12}O_{16}$, ($[\alpha]_D$ in alcohol = +135). Barium hydroxide, in presence of alcohol, precipitates $C_{18}H_{32}O_{16} \cdot BaO$ and ammoniacal lead subacetate, $C_{18}H_{24}Pb_4O_{16}$.

**Lactosinose.** — Lactosin. $C_{18}H_{32}O_{16}$?

*Occurrence.* — This sugar was discovered by Meyer* in the roots of *Silene vulgaris* and other Cariophyllaceæ. It has also been found in Quillai-bark (bark of *Quillaia Saponaria*) and in *Saponaria rubra.*

*Preparation.* The expressed juice of the roots of *Silene vulgaris* is treated with an excess of strong alcohol. The precipitate is dissolved in water, clarified with lead subacetate, the solution filtered and treated with ammoniacal lead acetate; the lead lactosinate is filtered off, decomposed in aqueous suspension with hydrogen sulphide, the solution filtered from lead sulphide and evaporated; the sirup thus obtained is treated with strong alcohol and the precipitated sugar, consisting of an amorphous mass, dried first over concentrated sulphuric acid and then at 110° C. The dried product is then boiled 1 to 3 days with 80 per cent alcohol under a reflux condenser; the quantity of alcohol should not be sufficient to dissolve all the crude sugar. Upon filtering the alcoholic extract and cooling, the lactosinose is deposited as crystals, which may be purified if necessary by recrystallization.

*Properties.* — Lactosinose, as above prepared, consists of small glistening crystals which, after drying over concentrated sulphuric acid, have a composition corresponding to $C_{18}H_{32}O_{16}$( or $C_{36}H_{64}O_{32}$). The sugar is easily soluble in water, somewhat soluble in 50 per cent alcohol and is dissolved by 350 parts of hot 80 per cent alcohol. The concentrated aqueous solution is very viscous. Lactosinose is strongly dextrorotatory, $[\alpha]_D^{16} = +211.7$. Upon drying at 110° C. lactosinose be-

* Ber., **17**, 685.

comes amorphous and in this condition shows a lower rotation, $[\alpha]_D = +168$ to $+190$.

Lactosinose is not affected by boiling solutions of dilute alkalies and does not reduce Fehling's solution except after long boiling (7 minutes) when a very slight reduction may take place. Upon oxidation with nitric acid a large amount of mucic acid is formed. Upon boiling a 1 per cent solution of the sugar with hydrochloric or sulphuric acid, using 1 part acid to 1 part sugar, lactosinose is slowly hydrolyzed, the specific rotation diminishing to below + 50. The products of the hydrolysis consist of about 45 per cent d-galactose; the presence of an undetermined dextrorotatory and of an undetermined levorotatory sugar is also indicated.

The compounds and other properties of lactosinose have not been investigated. More study is required upon lactosinose before its constitution and its exact relationship to other carbohydrates can be tabulated.

**Secalose.** — β-Levulin. $C_{18}H_{32}O_{16}$.

*Occurrence.* — Secalose, formerly called β-levulin, was discovered by Schulze and Frankfurt* in green rye (*Secale cereale*), where it occurs to the extent of 2 to 3 per cent. It has also been found in green oats and in ray-grass (*Lolium perenne*).

*Preparation.* — The alcoholic extract of green rye, or oats, is treated with strontium hydroxide solution and the strontium secalate, which is precipitated, filtered off, decomposed with carbon dioxide and the secalose precipitated from the evaporated filtrate by means of strong alcohol. Purification of the sugar is carried out in the same manner as described for stachyose.

*Properties.* — Secalose crystallizes as a hydrate in the form of white microscopic prisms, which upon heating in a stream of dry hydrogen at 100° C. lose all their water without decomposition. The anhydrous sugar has a composition corresponding to the formula $C_{18}H_{32}O_{16}$. The sugar is easily soluble in water, in which it exhibits levorotation, $[\alpha]_D = -28.6$ to $-31.7$. It does not reduce Fehling's solution.

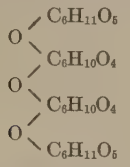
Secalose upon warming with dilute hydrochloric or sulphuric acid is rapidly hydrolyzed into d-fructose. No other sugar has been detected among the products of hydrolysis.

Additional investigation is required upon secalose before its constitution and its relationship to other sugars can be determined.

* Ber. **27**, 65, 3525.

## The Tetrasaccharides

### Tetrahexose Saccharides




**Stachyose.** — Mannatetrasaccharide. $C_{24}H_{42}O_{21} + 4\,H_2O$.

**Occurrence.** — The discovery of a tetrasaccharide by Tanret in ash manna has already been mentioned (p. 744). Tanret* showed later that this tetrasaccharide was identical with a sugar found by Planta† in the tubers of *Stachys tuberifera* and named by him stachyose. The sugar has also been found in the roots of different plants belonging to the Labiatæ, in the roots of *Lansium altuus* and in the white jasmine.

**Preparation.** — Stachyose according to Schulze and Planta‡ makes up from 14.16 to 73.07 per cent of the dry substance of the tubers of *Stachys tuberifera.*

To prepare the sugar the expressed juice of the tubers is clarified with lead subacetate and mercuric nitrate, the lead and mercury are precipitated from the filtrate by hydrogen sulphide and the clear solution neutralized with ammonium hydroxide, and evaporated to a sirup. The sirup thus prepared is poured into an excess of alcohol which throws down an abundant precipitate. The latter is separated, dissolved in a little water, clarified with phosphotungstic acid, filtered, the excess of clarifying agent removed with barium hydroxide solution, again filtered and saturated with carbon dioxide to remove barium; the barium carbonate is filtered off, the filtrate concentrated and again poured into alcohol which precipitates flakes of impure stachyose. The stachyose is purified by dissolving the flakes in water and precipitating with alcohol, repeating the process several times; the product is finally dissolved in a little water, alcohol added till the strength of the solution is 91 per cent; any precipitated stachyose is filtered off and saved and the filtrate set aside for crystallization, which usually requires several weeks' standing. If a little crystallized stachyose is available the process of crystallization may be hastened by priming.

**Properties.** — Stachyose is obtained as hard rhombic crystals of sweet taste and with a composition corresponding to the formula $C_{24}H_{42}O_{21} + 4\,H_2O$. The water of crystallization is partially removed

* Compt. rend., **136,** 1569. † Landw. Vers. Stationen, **25,** 473.
‡ Ber., **23,** 1692; **24,** 2705.

upon standing over concentrated sulphuric acid or upon warming to 100 °C. The water is completely removed at 115° to 120° C.; decomposition and oxidation set in, however, below this temperature so that the anhydride cannot be prepared in this way. The best method of dehydration is to heat the powdered sugar in a stream of dry hydrogen at 103° C. for half an hour; in this manner all water is removed without decomposition.

Stachyose is easily soluble in water, 1 part of the hydrate being dissolved by 0.75 parts water at 13° C.; at 15° C. the hydrate is soluble in 14 parts 60 per cent, in 55 parts 70 per cent and in 300 parts 80 per cent alcohol. It is insoluble in absolute alcohol.

Stachyose is strongly dextrorotatory, $[\alpha]_D$ for the anhydride $= +147.9$ to $+148.1$ (Schulze and Planta) and $+148.9$ (Tanret); $[\alpha]_D$ for the hydrate $= +132.75$ to $+133.85$ (Tanret) and $+133.5$ (Schulze). If $+148.5$ be taken for the anhydride, the theoretical $[\alpha]_D$ for $C_{24}H_{42}O_{21} + 4\,H_2O$ is $+134.0$.

Stachyose is not affected upon heating with dilute solutions of alkalies and does not reduce Fehling's solution. Upon oxidation with nitric acid stachyose yields 37 to 38 per cent mucic acid.

**Hydrolysis of Stachyose.** — Upon warming with acetic acid, or even upon prolonged boiling with water, stachyose is hydrolyzed into d-fructose and mannatrisaccharide.

$$\underset{\text{Stachyose}}{C_{24}H_{42}O_{21}} + H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{Mannatrisaccharide.}}{C_{18}H_{32}O_{16}}.$$

Upon warming with dilute hydrochloric or sulphuric acid stachyose is rapidly hydrolyzed into its component monosaccharides.

$$\underset{\text{Stachyose}}{C_{24}H_{42}O_{21}} + 3\,H_2O = \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{d-Glucose}}{C_6H_{12}O_6} + 2\,\underset{\text{d-Galactose.}}{C_6H_{12}O_6}.$$

The theoretical yield of reducing sugars from stachyose anhydride according to the preceding equation is 108.1 per cent; in actual practice, however, this yield is never reached owing to destruction of fructose. Winterstein* obtained as a maximum, after heating stachyose 1 hour with 2 per cent hydrochloric or sulphuric acid, only 80.14 per cent yield of reducing sugar which is less than 75 per cent of the theoretical.

**Fermentation.** — Stachyose is only partially fermented by yeast; invertase hydrolyzes the sugar into d-fructose and mannatrisaccharide, the former being quickly and the latter only slowly and imperfectly fermented.

**Lupeose.** — Lupeose, which was originally regarded as a galactan and afterwards as a disaccharide, is, according to the latest researches

* Landw. Vers. Stationen, **41**, 375.

of Schulze,* in all probability a tetrasaccharide. For want of other knowledge the sugar is placed in this class.

*Occurrence.* — Lupeose was discovered by Beyer† in lupine seeds but its preparation in a pure form was due first to Schulze‡ and his coworkers. The sugar occurs as a reserve substance in the seeds of *Lupinus luteus, L. angustifolius*, etc., and is completely metabolized during the first few days of germination.

*Preparation.* — Finely ground lupine seeds are extracted with 80 per cent alcohol and the filtered extract freed of impurities by successive treatments with tannic acid, lead acetate and phosphotungstic acid. After removing the excess of clarifying agents (see under stachyose) the solution is evaporated and treated with absolute alcohol. The precipitated lupeose is purified by dissolving in water and reprecipitating with alcohol as described under stachyose. The final product is dried over concentrated sulphuric acid.

*Properties.* — Lupeose consists of a white, amorphous, hygroscopic powder, which has not been obtained as yet in crystalline form. It is easily soluble in water, less soluble in 80 per cent alcohol, insoluble in absolute alcohol and ether. Lupeose is strongly dextrorotatory. According to the latest measurements of Schulze§ $[\alpha]_D = +148.0$. Lupeose is not affected by boiling solutions of dilute alkalies and does not reduce Fehling's solution. Oxidation with nitric acid gives a large yield of mucic acid. Upon boiling with dilute hydrochloric or sulphuric acid lupeose is hydrolyzed into a mixture consisting of d-galactose, d-fructose and d-glucose, the former to the extent of about 50 per cent. This would correspond to a tetrasaccharide made up of 2 molecules of d-galactose and 1 molecule each of d-glucose and d-fructose; additional investigation is required, however, before the composition of lupeose can be expressed with certainty.

**Verbascose.** — This sugar, discovered by Bourquelot and Bridel‖ in the roots of the common mullein (*Verbascum Thapsus*), has been classified provisionally as a tetrasaccharide.

*Preparation.* — Fresh mullein roots are extracted with boiling alcohol. The sugar is precipitated from the concentrated extract by barium hydroxide solution; the insoluble barium compound is filtered off, de-

* Ber., **43,** 2230.

† Landw. Vers. Stationen, **9,** 117; **14,** 164.

‡ Schulze and Steiger, Ber., **19,** 827; **20,** 280, Schulze and Winterstein, Ber., **25,** 2213.

§ Ber., **43,** 2233.

‖ Compt. rend. **151,760.**

composed in water with carbon dioxide, and the solution of sugar filtered; any excess of barium is removed with sulphuric acid. The filtered solution is concentrated and treated with a large excess of 95 per cent alcohol which causes a precipitation of the sugar. The latter is filtered off, and dried in vacuo over concentrated sulphuric acid. The sugar is purified by dissolving in hot methyl alcohol (diluted one-fifth with water), filtering and then adding one-half the volume of absolute alcohol. The verbascose crystallizes upon cooling.

*Properties.* — Verbascose is obtained as small needle-like crystals of sweetish taste, soluble in water, but almost insoluble in strong alcohol. The crystals, after drying in vacuo over concentrated sulphuric acid, lose 2.37 per cent of water of crystallization at 100° C. The sugar melts at 219° to 220° C. (Maquenne's Block) and at 213° C. (capillary tube). Verbascose is dextrorotatory, $[\alpha]_D$ (for the sugar dried at 100° C.) $= +$ 169.9, and does not reduce boiling Fehling's solution; it is only partially hydrolyzed by invertase and, upon oxidation with nitric acid, yields mucic acid equivalent to 56.7 per cent galactose; d-glucose and d-fructose are obtained as other products of hydrolysis. Verbascose is apparently a true isomer of stachyose from which it differs in higher melting point and in higher specific rotation.

# CHAPTER XXII

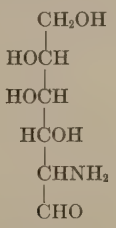
## THE AMINO SUGARS AND THE CYCLOSES

In addition to the monosaccharides, previously described, there are a number of closely related compounds which from their frequent association with the ordinary sugars and their similarity in properties have more than a theoretical interest for the analyst. Only two classes of substances will be considered in this connection, the amino sugars and the cyclic sugars; in the description of these only such compounds will be mentioned as may be met with in the investigation of plant and animal substances.

### The Amino Sugars

The amino sugars have considerable theoretical interest as they form one of the connecting links between the carbohydrates and the proteids. Only one compound, aminoglucose or d-glucosamine, will be described. For an account of the many synthetic amino sugars reference should be made to the special works upon the subject.*

**D-Glucosamine.** — Chitosamine.




**Occurrence.** — d-Glucosamine does not occur in nature, so far as known, in the free condition; it is formed, however, during the hydrolysis of many nitrogenous substances of animal and vegetable origin.

Among the animal substances which yield glucosamine upon hydrolysis the most important are the mucins or mucoids and the chitins. The mucin of human sputum yields upon hydrolysis with hydrochloric acid about 34 per cent of the weight of dry substance as glucosamine

* For a full description and bibliography of the amino sugars and carbohydrates, see article by Geza Zemplen in the Biochem. Handlexikon, p. 527.

chloride; mucins from other products of the body also yield large quantities of the same compound. Among the mucoids the ovomucoid of eggs, the chondromucoid of cartilage and the mucoid of blood serum have been examined and these yield in some cases as high as 30 per cent glucosamine chloride.

*Chitin.* — The material which yields the largest amount of glucosamine upon hydrolysis is chitin,* a nitrogenous substance found in the outer covering of lobsters, crabs, scorpions, spiders, insects and other members of the Arthropoda. Chitin is also found widely distributed in the vegetable kingdom, as a constituent of the cellular tissues and membranes of the lower orders of plants, such as lichens, mushrooms, moulds, fungi, bacteria, etc. Chitin, when purified, yields over 80 per cent of its weight in glucosamine chloride.

The exact chemical nature of chitin has not as yet been determined; it is also uncertain whether the chitins of different origins are identical in composition or are condensations of glucosamine with varying complexity. Araki† ascribed to the chitin of lobster shells the formula $C_{18}H_{30}N_2O_{12}$. Upon heating this with concentrated potassium hydroxide, acetic acid is split off with formation of chitosan.‡

$$\underset{\text{Chitin}}{C_{18}H_{30}N_2O_{12}} + 2\,H_2O = 2\,\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Chitosan.}}{C_{14}H_{26}N_2O_{10}}.$$

Chitosan is a yellow amorphous substance with pronounced basic properties; upon heating with concentrated hydrochloric acid to 110° C. it is rapidly hydrolyzed, yielding acetic acid and glucosamine chloride.

$$\underset{\text{Chitosan}}{C_{14}H_{26}N_2O_{10}} + 2\,HCl + 2\,H_2O = \underset{\text{Acetic acid}}{CH_3COOH} + 2\,\underset{\text{Glucosamine chloride.}}{\begin{array}{l}CH_2OH\\|\\(CHOH)_3\\|\\CH-NH_2HCl\\|\\CHO\end{array}}$$

According to Irvine§ the formula of chitin is $C_{30}H_{50}O_{19}N_4$, the hydrolysis with hydrochloric acid proceeding as follows:

$$\underset{\text{Chitin}}{C_{30}H_{50}O_{19}N_4} + 7\,H_2O + 4\,HCl = 4\,\underset{\text{Glucosamine chloride}}{C_6H_{13}O_5NHCl} + 3\,\underset{\text{Acetic acid.}}{CH_3COOH}$$

**Preparation of Glucosamine.**—Glucosamine chloride is most easily prepared from lobster shells; the latter are first pulverized and then washed in cold hydrochloric acid in order to remove mineral matter. The crude chitin thus obtained may be still further purified by warming with dilute alkalies and extracting with alcohol and ether. The ex-

* Discovered by Odier in 1823 (Mémoire, Soc. hist. natur. de Paris, **1,** 35).

† Z. physiol. Chem., **20,** 498.

‡ Hoppe-Seyler, Ber., **27,** 3329; **28,** 82.

§ J. Chem. Soc., **95,** 564–570 (1909).

tracted material is then heated to boiling with concentrated hydrochloric acid until solution is effected; the liquid is then diluted, decolorized with bone black, filtered and evaporated when the glucosamine chloride will separate as brilliant shining crystals. The compound is purified by recrystallizing from 80 per cent alcohol.

Glucosamine chloride has a sweet taste with a bitter after-flavor. Its solutions are strongly dextrorotatory, showing mutarotation; $[\alpha]_D$ after solution $= +100$ about and $[\alpha]_D$ constant $= +72.5$ (values given range from $+70$ to $+75$).

d-Glucosamine is liberated from its chloride by decomposing the latter in absolute alcohol with diethylamine, according to the method of Breuer,* or by treatment of the chloride with sodium methylate in absolute methyl alcohol according to the method of Lobry de Bruyn and van Ekenstein.† The presence or formation of water during the process must be excluded.

**Properties.**—Free d-glucosamine forms a fine white crystalline compound melting at about 110° C. with decomposition. It is stable in a dry atmosphere, but decomposes in presence of moisture with evolution of ammonia. It is easily soluble in water, forming an alkaline solution; it is also soluble in hot ethyl and methyl alcohols but insoluble in ether. d-Glucosamine is dextrorotatory, $[\alpha]_D = +44$ (Lobry de Bruyn) and $+47$ to $+50$ (Breuer). It is not fermented by yeast although readily attacked by moulds and bacteria.

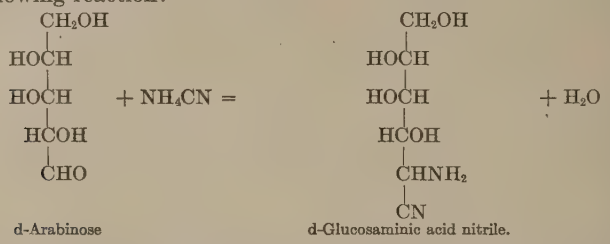
**Tests.**—d-Glucosamine or its chloride reduces Fehling's solution and other metallic salt solutions with great readiness, acting even in the cold. Warming with sodium hydroxide causes strong evolution of ammonia with rapid darkening of the solution and formation of caramel-like odors. d-Glucosamine upon careful oxidation with bromine is changed to d-glucosaminic acid which has the formula $CH_2OH \cdot (CHOH)_3 \cdot CHNH_2 \cdot COOH$. Oxidation with nitric acid causes a splitting off of the $NH_2$ group with formation of isosaccharic acid. Sodium amalgam and other reducing agents seem to have no action upon glucosamine. The ordinary color reactions of the aldose and ketose sugars also fail to develop.

d-Glucosamine gives a large number of derivatives and substitution products. Heated with phenylhydrazine the $NH_2$ group is split off and an osazone is formed which is identical in every respect with that of d-glucose and d-fructose. This reaction serves to establish the configuration of d-glucosamine.

**Synthesis of d-Glucosamine.** — The configuration of d-glucosamine has been confirmed by its synthesis from d-arabinose. Fischer

* Ber., **31**, 2193. † Ber., **31**, 2476.

and Leuchs* by treating d-arabinose with ammonium cyanide obtained the following reaction:



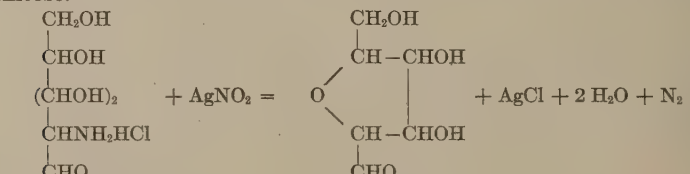

d-Arabinose — d-Glucosaminic acid nitrile.

The nitrile upon saponification yields d-glucosaminic acid, the lactone of which upon reduction is converted into d-glucosamine.

The above reaction may serve as a general example for the synthesis of amino sugars.

**Chitose.** — $C_6H_{10}O_5$.

*Preparation.* — d-Glucosamine chloride, when dissolved in water and shaken up in the cold with a slight excess of silver nitrite, loses its $NH_2$ group and by a process of inner condensation is converted into chitose.




d-Glucosamine chloride — Chitose.

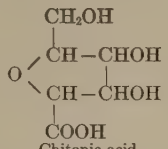
*Properties.* — Chitose has been obtained only as a colorless dextrorotatory non-fermentable sirup, all attempts to crystallize it having thus far proved unsuccessful. The above constitution, proposed by Fischer and Andreæ,† is based upon the reactions of chitose and upon the analysis of its derivatives.

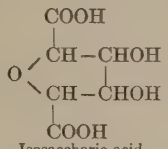
Chitose in many of its properties, such as reducing power, formation of hydrazones, oxime reaction, etc., behaves as an ordinary reducing sugar. On the other hand, in its failure to form osazones, chitose does not behave in a manner typical of the normal monosaccharides, and this is supposed to be due to the absence of a HCOH group in the position adjoining the CHO radical.

Chitose was first observed by Berthelot‡ in the action of mineral acids upon chitin. The chitose thus obtained seems to have been due, however, to the decomposition of glucosamine.

* Ber., **35**, 3787; **36**, 24. † Ber., **36**, 2587. ‡ Compt. rend., **47**, 227.

*Reactions.* — Chitose upon oxidation with bromine is converted into chitonic acid, $C_6H_{10}O_6$, and upon oxidation with nitric acid into isosaccharic acid, $C_6H_8O_7$. The configuration of these follows from that of chitose:

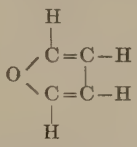

Chitonic acid


Isosaccharic acid.

Chitonic acid was obtained by Fischer and Tiemann* as a sirup ($[\alpha]_D = +44.5$), and isosaccharic acid as a white crystalline compound melting at 184° to 185° C. ($[\alpha]_D = +48$ about). The two acids do not form lactones and cannot be reduced by means of sodium amalgam.

Isosaccharic acid in presence of dehydrating agents is converted into dehydromucic acid and gives the characteristic reaction of this when heated with sulphuric acid and isatin (p. 781).

Chitose, chitonic and isosaccharic acids can be regarded as hydrated derivates of furfuran which has the formula



Their close relationship to furfural and its derivatives is referred to elsewhere (p. 782).

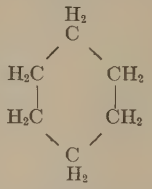
## The Cycloses

The cycloses† are an important group of compounds, widely distributed in nature and forming a connecting link between the sugars and the aromatic benzol-ring derivatives. The cycloses frequently occur in nature associated with the sugars and there seems to be an intimate physiological connection between the two groups of substances; the transformation of the one group into the other has not, however, been accomplished as yet in the laboratory. Although a number of the cycloses are isomeric with several of the sugars, the cycloses are not sugars in the chemical sense, as they contain no aldehyde or ketone group and give none of the characteristic sugar reactions.

* Ber., **27**, 138.

† For a full description and bibliography of the cycloses see article by Viktor Grafe in the Biochemisches Handlexikon, p. 551.

The cycloses may be regarded chemically as derivatives of hexamethylene, or hexahydrobenzol, which is a cyclic carbon compound of the formula:

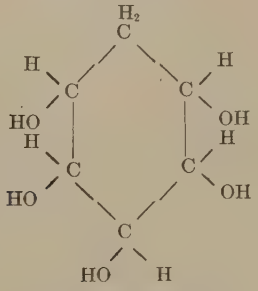


**Betite,** $C_6H_8(OH)_4$. — A compound answering to the properties of a tetroxyhexamethylene was found by Lippmann* in the end products of beet molasses and was hence given the name of *betite*.

Betite crystallizes in colorless prisms melting at 224° C. It is easily soluble in water and is slightly dextrorotatory. It has no reducing power, is not attacked by boiling alkalies and upon oxidation yields quinone.

**QUERCITE.** — Acorn sugar. Oak sugar.

$C_6H_7(OH)_5$.



Pentoxyhexamethylene

Quercite, which is isomeric with the methylpentoses, $C_6H_{12}O_5$, is widely distributed in nature, being found in acorns, cork, bark and other tissues of the oak. Of the large number of possible isomeric pentoxyhexamethylenes quercite is the only one at present known.

Quercite was discovered by Braconnot;† it is best prepared by extracting finely ground acorns with cold water. The filtered extract is evaporated in vacuum at 40° C. and any sugars which are present destroyed by fermentation with yeast; the solution is then clarified by means of lead subacetate to remove tannic acid and other impurities

* Ber., **34**, 1159. † Ann. chim. phys. [3], **27**, 392.

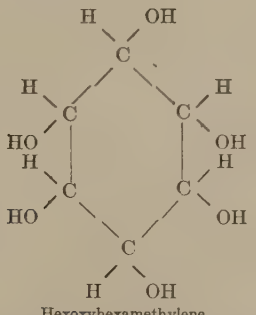
and the filtrate freed from excess of lead by means of hydrogen sulphide. The clear filtered solution upon evaporation gives crystals of quercite which are purified by recrystallizing from alcohol.

**Properties.** — Quercite crystallizes in colorless monoclinic prisms which melt at 234° C., dissolve in 8 to 10 parts of water and have a sweet taste. It is soluble in hot alcohol but insoluble in ether. Quercite is dextrorotatory, $[\alpha]_D^{16} = +\ 24.24$. It is not fermented by yeast, although certain bacteria are able to effect a slow decomposition.

**Tests.** — Quercite does not reduce Fehling's solution and fails to give any of the reactions characteristic of the sugars. Hot solutions of the alkalies are without action. Upon heating at 260° to 290° C., quercite is decomposed into quinone, $C_6H_4O_2$, hydroquinone, $C_6H_4(OH)_2$, pyrocatechin, $C_6H_4(OH)_2$, and pyrogallol, $C_6H_3(OH)_3$, which sublime with other benzol derivatives. A similar series of compounds is obtained upon heating with concentrated hydriodic acid or fusing with potassium hydroxide.

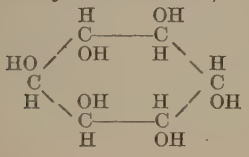
Quercite having 5 OH groups yields a corresponding number of acetates upon heating with acetic anhydride at temperatures ranging from 100° to 150° C.

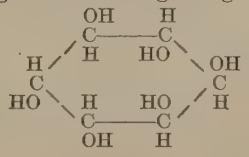
**The INOSITES.** — $C_6H_6(OH)_6$.




Hexoxyhexamethylene

*Isomeric Forms.* — The inosites, which are isomeric with the hexoses, $C_6H_{12}O_6$, are widely distributed in both the vegetable and the animal worlds. Of the nine possible arrangements of the H and OH groups of inosite upon the two sides of the ring plane only two of these arrangements possess molecular assymetry and there would, therefore, be only two optically active isomers, corresponding to the following configurations:



The two optically active d- and l inosites corresponding to the above configurations occur in nature as their methyl esters pinite and quebrachite from which they have been separated by treatment with hydriodic acid.

A peculiarity of the inosites is that none of their carbon atoms is structurally asymmetric, two bonds of each C atom being connected alike with reference to the remainder of the ring; this apparent exception to the theory of van't Hoff and Le Bel disappears, however, if the question is regarded from the standpoint of molecular assymmetry.

**d-Inosite,** $C_6H_{12}O_6$. — This compound has not been found as yet free in nature; its methyl ester, however, is widely distributed as pinite, and d-inosite is obtained directly from this by heating with concentrated hydriodic acid. The reaction proceeds quantitatively as follows:

$$\underset{\text{Pinite}}{C_6H_6(OH)_5(OCH_3)} + \underset{\substack{\text{Hydriodic}\\ \text{acid}}}{HI} = \underset{\text{d-Inosite}}{C_6H_6(OH)_6} + \underset{\text{Methyl iodide.}}{CH_3I}.$$

*Properties.* — d-Inosite consists of small colorless octahedral crystals which melt at 247° to 248° C., and are easily soluble in water, less soluble in alcohol, but insoluble in ether. By crystallizing from water a hydrate has been obtained having the formula $C_6H_{12}O_6 + 2\,H_2O$. d-Inosite is dextrorotatory without mutarotation, $[\alpha]_D = +\,65$; it is not fermented by yeast, and does not reduce Fehling's solution.

*Tests.* — All of the inosites upon oxidation with nitric acid yield colored oxyquinone derivatives. In carrying out this test the method of Scherer* is generally used. A small quantity of the material to be tested is treated with a little nitric acid and evaporated upon the water bath almost to dryness; a little ammoniacal barium chloride or calcium chloride solution is then added and the solution again evaporated. If inosite is present a beautiful rose red color will develop; 0.5 mg. of inosite may be detected in this way. Seidel† has modified this test by using ammoniacal strontium acetate to develop the color and in this way 0.3 mg. of inosite may be detected.

d-Inosite when heated to boiling with an excess of acetic anhydride in presence of a little zinc chloride is converted into the hexacetate $C_6H_6(CH_3COO)_6$, which is obtained as an amorphous mass insoluble in water but soluble in alcohol ($[\alpha]_D = +\,9.75$).

**Pinite,** $C_6H_6(OH)_5(OCH_3)$. — This, the methyl ester of d-inosite, is isomeric with the methylhexose sugars and is found widely distributed in nature. It was discovered by Berthelot‡ in 1856 in the

* Ann., **73,** 322; **81,** 375. † Chem. Ztg., **11,** 676.
‡ Compt. rend., **41,** 392.

resin of the *Pinus lambertiana* of California; it also occurs as sennite* in Senna leaves, as matezite† in the juice of the Madagascar rubber plant (*Mateza roritina*) and has also been found in the mother liquors‡ from the crystallization of coniferin. The identity of these various methyl esters of d-inosite with pinite has been established by Combes,§ Wiley,‖ and others.¶

Pinite forms white rhombic-hemihedral crystals melting at 185° to 186° C., and subliming without decomposition at 200° C. It has the same degree of sweetness as cane sugar, is easily soluble in water, less soluble in alcohol and insoluble in ether. It is not fermentable, and does not reduce Fehling solution. Pinite is dextrorotatory, $[\alpha]_D = +65.5$.

**l-Inosite,** $C_6H_{12}O_6$. — This compound has been found as yet only in the form of its methyl ester, quebrachite, from which it was obtained by Tanret** upon heating with hydriodic acid. The reaction is the same as that given for pinite.

*Properties.* — l-Inosite crystallizes from alcohol as the anhydride $C_6H_{12}O_6$ in the form of colorless prisms melting at 247° C. A hydrate, $C_6H_{12}O_6 + 2\ H_2O$, has been obtained by crystallizing from water. l-Inosite is easily soluble in water, less soluble in alcohol but insoluble in ether. It is levorotatory, $[\alpha]_D = -65$ for the anhydride without mutarotation, is unfermentable and does not reduce Fehling's solution.

l-Inosite gives Scherer's inosite reaction upon heating with nitric acid. With acetic anhydride an amorphous hexacetate is formed; the compound is levorotatory ($[\alpha]_D = -10$) but in other respects behaves the same as the hexacetate of d-inosite.

**Quebrachite,** $C_6H_6(OH)_5(OCH_3)$. — This, the methyl ester of l-inosite, occurs in the bark of the Quebracho tree. It crystallizes in prisms melting at 186° to 187° C.; the crystals are very sweet, easily soluble in water, less soluble in alcohol and insoluble in ether. Quebrachite is levorotatory, $[\alpha]_D = -80$; this figure, though of opposite sign, is not of the same value as that of pinite ($+65.5$), so that the two compounds are not optical antipodes. The compound is not attacked by dilute alkalies or acids; heated with concentrated nitric acid it gives Scherer's reaction. Quebrachite is unfermentable and does not reduce Fehling's solution.

* Dragendorff and Kubly, Ztschr. f. Chemie (1866), 411.
† Girard, Compt. rend., **77,** 995; **110,** 84.
‡ Tiemann and Haarmann, Ber., **7,** 609.
§ Compt. rend., **110,** 46.
‖ Amer. Chem. Jour., **13,** 228.
¶ See Maquenne's "Les Sucres," p. 209.
** Compt. rend., **109,** 908.

**d, l-Inosite.** — Racemic inosite was obtained by Maquenne and Tanret* by dissolving and crystallizing equal parts of d- and l-inosite. The anhydride consists of colorless crystals melting at 253° C.; the substance behaves as a true acemic combination and not as a simple mixture. d, l-Inosite is optically inactive; in its chemical behavior it reacts the same as either d- or l-inosite. It is not fermented by yeast; it has been partially resolved by Tanret who found that *Aspergillus niger* at low temperatures caused the inactive solution to become sensibly levorotatory.

**i-Inosite,** $C_6H_{12}O_6$ (Phaseomannite, Nucite, Dambose). *Occurrence.* — Inactive inosite, also called anti- or mesoinosite, is the only inosite which has thus far been found free in nature. It was discovered by Scherer† in 1850 in the mother liquors from a preparation of creatine obtained by extracting meat, and has since been found to be very widely distributed throughout the animal and vegetable kingdoms. It occurs in the muscles, kidneys, liver, lungs, heart, brain and other organs of the body and has also been found in the urine of patients afflicted with Bright's disease and diabetes, and also frequently in normal urines. The occurrence of inosite in the urine is sometimes termed inosuria.

In the vegetable world i-inosite has been found in green beans, peas and other legumes, in the cabbage, in the leaves of asparagus, the potato, dandelion, grape vine, oak, ash and other trees, in different mushrooms, in the roots of many plants and in the juices of grapes, blueberries and other fruits.

In the combined form i-inosite occurs as its methyl esters *bornesite* and *dambonite.*

*Preparation.* — i-Inosite is prepared from meat by first extracting the finely cut material with water. The aqueous extract is then slightly acidified with acetic acid and boiled; the coagulated albumin is filtered off and the filtrate clarified with normal lead acetate. The solution is again filtered and the filtrate heated with an excess of lead subacetate solution and allowed to stand for 1 to 2 days. The basic lead-inosite compound is filtered off and decomposed in water with hydrogen sulphide. The filtrate from the lead sulphide is concentrated, treated with an excess of hot alcohol and the solution separated from any precipitated impurities. The alcoholic solution upon cooling will usually deposit crystals of inosite; if no crystals form, the separation may be promoted by adding ether to the point of turbidity, and setting

* Compt. rend., **110,** 86.

† Ann., **73,** 322.

the solution aside in a cool place. The compound is purified by recrystallizing from alcohol.

To prepare inosite from plant materials the process employed by Maquenne* for its extraction from walnut leaves may be employed. The dried finely ground leaves are extracted repeatedly with 5 to 6 parts of boiling water, the residue pressed out and the brownish colored extract treated hot with concentrated milk of lime until the precipitate which has formed settles quickly. The solution is filtered and the filtrate treated with a very slight excess of normal lead acetate. The solution is again filtered and the inosite precipitated with ammoniacal lead subacetate solution. The precipitate, which should be perfectly white, is filtered off and then decomposed in aqueous suspension with hydrogen sulphide. The filtrate from the lead sulphide precipitate is evaporated to a sirup; the latter is then treated while still warm (about 50° C.) with 7 to 8 per cent of its volume of concentrated nitric acid which oxidizes most of the impurities but is without action upon the inosite. (Excess of acid and high temperature must, however, be avoided.) The acid solution is then heated for a few minutes upon the water bath and then treated with 4 to 5 volumes of strong alcohol; after cooling 1 volume of ether is added when the inosite will begin to crystallize. After 24 hours the solution is decanted, the impure inosite washed with alcohol and then recrystallized from acetic acid. To remove the last traces of coloring matter, calcium sulphate and other impurities, the inosite is dissolved in water and treated with a slight excess of barium hydroxide solution. The solution is filtered, the excess of barium removed with ammonium carbonate and the clear filtrate evaporated to dryness. The residue upon recrystallizing from water gives pure inosite. By this method Maquenne obtained 440 gms. of inosite from 150 kgs. of leaves, a yield of about 0.29 per cent.

*Properties.* — i-Inosite crystallizes from alcohol or from water above a temperature of 50° C. as the anhydride in the form of needles melting at 224° C. Upon crystallizing from water below a temperature of 50° C., the hydrate $C_6H_{12}O_6 + 2\,H_2O$ is obtained in the form of large hexagonal monoclinic crystals which effloresce rapidly in a dry atmosphere. i-Inosite has a sweet taste, is very soluble in water (7.5 parts at 15° C. for the anhydride), less soluble in alcohol and insoluble in ether. It is optically inactive even after the addition of borax; its optical neutrality is not affected by the attack of moulds as is the case with d, l-inosite. It is not fermented by yeast, although certain bacteria appear to cause destructive changes. It does not reduce Fehling's

* "Les Sucres," p. 216.

reagent, although it produces a metallic mirror with ammoniacal silver solution. i-Inosite gives Scherer's reaction, described under d-inosite.

**Bornesite.** $C_6H_6(OH)_5(OCH_3)$. — This, the monomethyl ester of i-inosite, was discovered by Girard* in crude Borneo caoutchouc; it was also found by Flint and Tollens† in the wash waters from certain rubber factories. It is isomeric with pinite and quebrachite and crystallizes in rhombic prisms melting at about 200° C. and subliming at 205° C. It is easily soluble in water, but less soluble in alcohol. It is dextrorotatory, $[\alpha]_D = +32$ (Girard), $+31.16$ (Flint and Tollens); it is unfermentable and does not reduce Fehling's solution. It is decomposed‡ by heating with hydriodic acid into methyl iodide and i-inosite.

**Dambonite,** $C_6H_6(OH)_4(OCH_3)_2$. — This, the dimethyl ester of i-inosite, was discovered by Girard§ in Gabon rubber; it has also been found in the latex or milky caoutchouc yielding juice of the *Castilloa elastica*. Dambonite crystallizes in white rhombic prisms which melt at about 190° to 195° C. and sublime between 200° to 210° C. It is sweet, very soluble in water and dilute alcohol, unfermentable, optically inactive and does not reduce Fehling's solution. Dambonite forms with potassium iodide a double salt of the formula $C_8H_{16}O_6KI$. Upon heating with hydriodic acid it yields methyl iodide and i-inosite. Hydrolysis is also effected upon heating with concentrated hydrochloric acid.

**Quercinite,** $C_6H_6(OH)_6$. — This compound was discovered by Vincent and Delachanal‖ in the mother liquors obtained from the crystallization of quercite. Quercinite crystallizes from cold water as a hydrate, the crystals of which effloresce rapidly upon exposure to the air. Crystallized from hot water the anhydride is obtained in the form of rhombic prisms melting at 340° C. The anhydride is soluble in 66 parts of cold water, easily soluble in hot water, insoluble in alcohol and ether; it is optically inactive, unfermentable and does not reduce Fehling's solution. Quercinite gives Scherer's inosite reaction, and in its general behavior seems to belong to the group of inactive inosites of which there are seven possible stereo-isomers.

**Phytin.** — Inosite also exists in nature in combination with phosphoric acid as phytin, the principal phosphorus compound of vegetable

* Compt. rend., **73,** 426; **77,** 995.
† Ann., **272,** 288.
‡ Maquenne, Ann. chim. phys. [6], **12,** 566.
§ Compt. rend., **67,** 820.
‖ Compt. rend., **104,** 1855.

seeds. Phytin, according to the researches of Suzuki, Yoshimura and Takaishi,* is an inosite-hexaphosphoric acid $C_6H_6[OPO(OH)_2]_6$, which, by the action of a special enzyme *phytase*, is hydrolyzed into inosite and phosphoric acid.

$$\underset{\text{Phytin}}{C_6H_6[OPO(OH)_2]_6} + 6\,H_2O = \underset{\text{Inosite}}{C_6H_{12}O_6} + 6\,\underset{\text{Phosphoric acid}}{H_3PO_4}.$$

* Bull. College of Agric., Tokyo, **7**, 495, 503 (1907).

# CHAPTER XXIII

## THE SUGAR ALCOHOLS AND SUGAR ACIDS

The close relationship of the sugars to the alcohols upon the one side and to the monobasic and dibasic acids upon the other has already been mentioned. While these two groups of substances are entirely distinct from the sugars, their constant association with the sugars in nature and their great importance in many analytical and synthetical operations of sugar chemistry are of sufficient account to require brief mention.

### The Sugar Alcohols

Of some thirty known sugar alcohols the following eight have been found in nature: glycerol, erythrite, adonite, sorbite, mannite, dulcite, perseite and volemite. Reference has already been made to the occurrence of these.

**Synthesis of the Sugar Alcohols.** — The sugar alcohols are generally prepared by the action of nascent hydrogen upon an aldose or ketose sugar. The reduction is best accomplished by means of sodium amalgam. The process of Fischer* is as follows: a 10 per cent aqueous solution of the sugar is treated ice cold with small additions of sodium amalgam (2 to $2\frac{1}{2}$ per cent sodium content) until the reducing power of the solution has almost disappeared. During the first part of the operation the solution is kept weakly acid with constant additions of dilute sulphuric acid in order to prevent molecular transformation of sugar by action of the free alkali; in the last stages of the reduction the solution is kept faintly alkaline. After reduction the solution is neutralized, evaporated until sodium sulphate begins to crystallize and then poured into 8 volumes of absolute alcohol. The alcoholic solution is filtered from sodium sulphate and evaporated when the sugar alcohol is obtained either as a sirup or in crystalline form.

**Formation of Sugar Alcohols During Fermentation.** — The sugar alcohols are also formed in many anaërobic fermentations through a similar process of reduction. The best-known example of this is the so-called mannitic fermentation which takes place frequently in the juices of the sugar cane, sugar beet, grapes, apples and in other vege-

* " Untersuchungen über Kohlenhydrate " (1909), pp. 186, 292, 473, etc.

table extracts. The sugar is changed partly to mannite and partly to the mucilaginous gum dextran $(C_6H_{10}O_5)_n$; the latter can be precipitated by means of alcohol and the mannite obtained by evaporation of the alcoholic solution. The presence of mannite in wines, musts, vinegars, sugar-house products, distillery residues, etc., is due largely to the result of such fermentations.

**Properties and Reactions of the Sugar Alcohols.** — The sugar alcohols resemble one another in their sweet taste, in not being fermented by yeast and in the complete lack of the aldehyde or ketone properties (reduction of Fehling's solution, hydrazone and osazone formation, color reactions, etc.), characteristic of the parent sugar. In presence of free alkalies the sugar alcohols give soluble complex substitution products with many of the heavy metals; for this reason salts of copper, etc., are not precipitated by alkaline hydroxides in presence of glycerol, mannite and other polyvalent alcohols. This property, however, is not a characteristic one, being also shared by the sugars and their acid derivatives.

*Compounds of Sugar Alcohols and Metals.* — If excess of alkali be avoided, the metallic substitution products of the sugar alcohols may be obtained in some cases as a precipitate. Mannite, for example, can be precipitated from solution in presence of copper sulphate by adding ammonium hydroxide to faintest possible excess. The blue copper-mannite compound can then be filtered off; it is practically insoluble in water, but is soluble in excess of ammonia from which solution the mannite can be regenerated after removing the copper with hydrogen sulphide. This process due to Guignet * can be utilized for the separation of mannite from plant juices.

*Reaction of Sugar Alcohols with Borax.* — The behavior of many sugar alcohols with borax and boric acid is also worthy of mention. If a little borax be added to an aqueous solution of mannite, arabite, etc., the solution becomes strongly acid, with a marked increase in the electrical conductivity.† The phenomenon is due to the formation of alcohol-boric acid complexes, the constitution of which remains in doubt. The acid complex, which is strong enough to decompose carbonates, undergoes dissociation ‡ upon dilution with water.

Borax and boric acid also have the peculiar property of intensifying the rotatory power § of solutions of the sugar alcohols to a very marked

* Compt. rend., **109,** 528.
† Magnanini, Gazetta chim. Ital., **20,** 428.
‡ Klein, Bull. soc. chim. [2], **29,** 195, 198, 357.
§ Vignon, Compt. rend., **77,** 1191; **78,** 148.

degree, the result no doubt of the higher specific rotation of the boric acid alcohol complex. Acid molybdates* of sodium and ammonium produce the same effect to an even greater extent; so also the tungstate† and paratungstate of sodium. Polarization of solutions before and after the addition of constant quantities of borax has been employed for estimating certain sugar alcohols, as mannite,‡ in mixture with other substances.

Table CIII gives a list of the different alcohols, with a few of their properties, which are obtained by reduction of the different monosaccharides. The sugar alcohols, which have been found free in nature, are marked in italics. In the nomenclature of the sugar alcohols the ending *-ite* § is usually substituted in place of the termination *-ose* of the sugar, as pentite, hexite, etc.

It will be noted from the table that the ketose sugars, erythrulose, fructose, sorbose, and tagatose, yield two isomeric alcohols upon reduction. This necessarily follows from the configuration, since reduction of the $\begin{matrix}|\\ C=O \\ |\end{matrix}$ group will give both $\begin{matrix}|\\ HCOH \\ |\end{matrix}$ and $\begin{matrix}|\\ HOCH \\ |\end{matrix}$ isomers.

**Reaction of Sugar Alcohols with Aldehydes.** — A number of reactions, which have been employed for the separation and identification of the sugar alcohols, should be mentioned. Chief among these are the reactions with formaldehyde, acetaldehyde and benzaldehyde in presence of strong hydrochloric or sulphuric acid (50 per cent) with formation of a characteristic group of compounds known as acetals.

*Formals.* — Mannite, for example, when heated with equal parts of 40 per cent formaldehyde and concentrated hydrochloric acid gives mannite triformal, ‖ $C_6H_8O_6(CH_2)_3$, which consists of white needles, only slightly soluble in water and melting at 227° C.

*Acetals.* — In the same way, by heating mannite with acetaldehyde or paracetaldehyde in presence of concentrated hydrochloric acid or 50 per cent sulphuric acid, mannite triacetal, $C_6H_8O_6(C_2H_4)_3$, is formed.

*Benzals.* — Of greater value than the formals and acetals for separation and identification of the sugar alcohols are the benzals. This re-

* Gernez, Compt. rend., **112,** 1360.

† Klein, Compt. rend., **89,** 484.

‡ Muller, Bull. soc. chim. [3], **11,** 329.

§ Many chemists prefer the ending *-itol* in place of *-ite*, as mannitol, arabitol, perseitol, etc.; while this conforms with the rule that all alcohols should end in *-ol* the author has preferred the older and simpler terminology, which is still retained by Fischer, Tollens, Lippmann, Maquenne and other leading authorities.

‖ Tollens and Schulz, Ber., **27,** 1892.

TABLE CIII

*Classification and Properties of the Sugar Alcohols*

| Sugar. | Alcohol. | | Properties of Alcohol. | | |
|---|---|---|---|---|---|
| | Name. | Formula. | Appearance. | Melting point. Centigrade. | Specific rotation, $[\alpha]_D$. |
| Glycolose, $C_2H_4O_2$ | Glycol | $C_2H_6O_2$ | Thick sirup | ..... | 0 |
| Glycerose, $C_3H_6O_3$ | *Glycerol* | $C_3H_8O_3$ | Thick sirup | ..... | 0 |
| Dioxyacetone, $C_3H_6O_3$ | *Glycerol* | $C_3H_8O_3$ | | | |
| l-Erythrose, $C_4H_8O_4$ | *i-Erythrite* | $C_4H_{10}O_4$ | White prisms | 126° | 0 |
| d-Erythrose, $C_4H_8O_4$ | *i-Erythrite* | $C_4H_{10}O_4$ | | | |
| l-Threose, $C_4H_8O_4$ | l-Erythrite | $C_4H_{10}O_4$ | White rhomb. prisms | 88° | + 4.3° in water<br>−10.5° in 90% alcohol |
| d-Threose, $C_4H_8O_4$ | d-Erythrite | $C_4H_{10}O_4$ | White rhomb. prisms | 88° | − 4.4° in water<br>+10.1° in 90% alcohol |
| d,l-Threose | d,l-Erythrite | $C_4H_{10}O_4$ | White silky needles | 72° | 0 |
| d-Erythrulose, $C_4H_8O_4$ | i-Erythrite | $C_4H_{10}O_4$ | | | |
| | d-Erythrite | $C_4H_{10}O_4$ | | | |
| Methylerythrose, $CH_3C_4H_7O_4$ | Methylerythrite | $CH_3C_4H_9O_4$ | | | |
| l-Arabinose, $C_5H_{10}O_5$ | l-Arabite | $C_5H_{12}O_5$ | White prism. crystals | 102° | 0 in water<br>−5.3° with borax |
| d-Arabinose, $C_5H_{10}O_5$ | d-Arabite | $C_5H_{12}O_5$ | White prism. crystals | 103° | +7.7° with borax |
| l-Xylose, $C_5H_{10}O_5$ | Xylite | $C_5H_{12}O_5$ | Sirup | ..... | 0 in $H_2O$<br>0 with borax |
| d-Xylose, $C_5H_{10}O_5$ | Xylite | $C_5H_{12}O_5$ | | | |
| d-Lyxose, $C_5H_{10}O_5$ | d-Arabite | $C_5H_{12}O_5$ | | | |
| l-Ribose, $C_5H_{10}O_5$ | *Adonite* | $C_5H_{12}O_5$ | White prism. needles | 102° | 0 |
| Rhamnose, $CH_3C_5H_9O_5$ | Rhamnite | $CH_3C_5H_{11}O_5$ | White prism. crystals | 121° | +10.7° in water |
| d-Glucose, $C_6H_{12}O_6$ | *d-Sorbite* | $C_6H_{14}O_6$ | Fine white needles | 55° (1 mol. $H_2O$)<br>75° ($\frac{1}{2}$ mol. $H_2O$)<br>110° (anhyd.) | −1.73° in water<br>+1.5° with borax |
| d-Mannose, $C_6H_{12}O_6$ | *d-Mannite* | $C_6H_{14}O_6$ | Fine white needles | 166° | −0.25° in water<br>+22.5° with borax |
| l-Mannose, $C_6H_{12}O_6$ | l-Mannite | $C_6H_{14}O_6$ | Fine white needles | 166° | Levorotatory with borax |
| d,l-Mannose | d,l-Mannite | $C_6H_{14}O_6$ | White prism. crystals | 170° | 0 |
| d-Gulose, $C_6H_{12}O_6$ | *d-Sorbite* | $C_6H_{14}O_6$ | Fine white needles | | |
| l-Gulose, $C_6H_{12}O_6$ | l-Sorbite | $C_6H_{14}O_6$ | Fine white needles | 75° ($\frac{1}{2}$ mol. $H_2O$) | −1.4° with borax |
| d-Idose, $C_6H_{12}O_6$ | d-Idite | $C_6H_{14}O_6$ | Sirup | | |

TABLE CIII (*concluded*)

| Sugar. | Alcohol. | | Properties of Alcohol. | | |
|---|---|---|---|---|---|
| | Name. | Formula. | Appearance. | Melting point. Centigrade. | Specific rotation, $[\alpha]_D$. |
| l-Idose, $C_6H_{12}O_6$ | l-Idite | $C_6H_{14}O_6$ | White rhomb. crystals | 73.5° | +3.5 in water |
| d-Galactose, $C_6H_{12}O_6$ | *Dulcite* | $C_6H_{14}O_6$ | White rhomb. prisms | 188° | 0 in water<br>0 with borax<br>0 with molybdate |
| l-Galactose, $C_6H_{12}O_6$ | *Dulcite* | $C_6H_{14}O_6$ | | | |
| d-Talose, $C_6H_{12}O_6$ | d-Talite | $C_6H_{14}O_6$ | White crystals | 86° | +3.05° in water<br>−0.55° with borax<br>+60° with molybdate |
| d,l-Talose, $C_6H_{12}O_6$ | d,l-Talite | $C_6H_{14}O_6$ | White needles | 67° | 0° |
| d-Fructose, $C_6H_{12}O_6$ | *d-Mannite*<br>*d-Sorbite* | $C_6H_{14}O_6$<br>$C_6H_{14}O_6$ | | | |
| d-Sorbose, $C_6H_{12}O_6$ | *d-Sorbite*<br>d-Idite | $C_6H_{14}O_6$<br>$C_6H_{14}O_6$ | | | |
| l-Sorbose, $C_6H_{12}O_6$ | l-Sorbite<br>l-Idite | $C_6H_{14}O_6$<br>$C_6H_{14}O_6$ | | | |
| d-Tagatose, $C_6H_{12}O_6$ | *Dulcite*<br>d-Talite | $C_6H_{14}O_6$<br>$C_6H_{14}O_6$ | | | |
| Rhamnohexose $CH_3C_6H_{11}O_6$ | Rhamnohexite | $CH_3C_6H_{13}O_6$ | White prisms | 173° | +14° |
| Glucoheptose, $C_7H_{14}O_7$ | Glucoheptite | $C_7H_{16}O_7$ | White prisms | 128° | 0° |
| d-Mannoheptose, $C_7H_{14}O_7$ | *Perseite* | $C_7H_{16}O_7$ | White needles | 188° | −1.2° in water<br>+4.75° with borax<br>+48° with molybdate |
| l-Mannoheptose, $C_7H_{14}O_7$ | l-Mannoheptite | $C_7H_{16}O_7$ | White needles | 187° | Slightly + |
| Galaheptose, $C_7H_{14}O_7$ | Galaheptite | $C_7H_{16}O_7$ | White needles | 184° | −4.35° with borax |
| Volemose, $C_7H_{14}O_7$ | *Volemite* | $C_7H_{16}O_7$ | White needles | 151° | +1.92° in water<br>+22° with borax |
| Glucooctose, $C_8H_{16}O_8$ | Glucooctite | $C_8H_{18}O_8$ | White needles | 141° | +2° in water<br>+6° with borax |
| Mannooctose, $C_8H_{16}O_8$ | Mannooctite | $C_8H_{18}O_8$ | Fine micro. cryst. | 258° | |
| Galaoctose, $C_8H_{16}O_8$ | Galaoctite | $C_8H_{18}O_8$ | Fine needles | 225° | |
| Glucononose, $C_9H_{18}O_9$ | Glucononite | $C_9H_{20}O_9$ | Small prisms | 194° | |

action, which is due to Meunier,* has been much employed by Fischer.† The alcohol, in concentrated hydrochloric acid or 50 per cent sulphuric acid, is shaken up with benzaldehyde when the benzal derivatives of erythrite, xylite, mannite, sorbite, and perseite will quickly precipitate: the separation with these alcohols is almost quantitative. In the case of glycerol, arabite, and dulcite the benzal derivatives obtained by this method remain in solution so that no separation is effected.

As to the constitution of the benzals obtained by the method just described there appears to be no uniformity. Mannite, for example, combines with three molecules of benzaldehyde; erythrite, xylite, adonite, sorbite, and perseite with two; and glucoheptite with only one. This peculiarity is probably due to the spatial arrangement of the alcohol groups within the molecule, although no satisfactory theory ‡ has as yet been formulated. As in the case of the formals and acetals the reaction probably results from the withdrawal of the H from 2 hydroxyl groups of the sugar alcohol by the O of the aldehyde. The reaction, for example, with sorbite would be:

$$\underset{\text{Sorbite}}{C_6H_{14}O_6} + 2\,O:\underset{\text{Benzaldehyde}}{\overset{C_6H_5}{\overset{|}{C}}-H} = \underset{\text{Sorbite-dibenzal}}{C_6H_{10}O_6\left(:\overset{C_6H_5}{\overset{|}{C}}-H\right)_2} + \underset{\text{Water.}}{2\,H_2O},$$

but which of the hydroxyl groups of the sugar alcohol participate in the reaction is not at present known.

The formulæ and properties of the more important benzal derivatives of the sugar alcohols are given in Table CIV.

The benzals upon boiling with 5 per cent sulphuric acid are decomposed into benzaldehyde and the free alcohol. The process of decomposition is much facilitated by the addition of a little free benzaldehyde. In a few cases, as with mannite, long boiling and a high temperature of heating are required to effect complete hydrolysis. The benzaldehyde can be removed by shaking out the cold acid solution with ether and the sulphuric acid eliminated by neutralizing with barium hydroxide and filtering off the barium sulphate. The clear filtrate upon evaporation will then yield the sugar alcohol either as a sirup or in the crystalline form. By this means it is possible to effect the separation of different sugar alcohols from plant extracts, juices, etc.

Sugar alcohols can be detected in the presence of sugars by first heating the solution with dilute hydrochloric acid to invert any higher saccharides; the sugars are then precipitated in the neutralized solution as osazones by means of phenylhydrazine. After filtering off the osa-

* Compt. rend., **106,** 1425, 1732; **107,** 910; **108,** 408. † Ber., **27,** 1524.
‡ See Fischer's discussion upon this point, Ber., **27,** 1524.

zones, the filtrate is shaken out with ether to remove excess of phenylhydrazine and the aqueous solution tested for sugar alcohols with benzaldehyde in the manner described.

TABLE CIV

*Giving Formulæ and Properties of Sugar Alcohol Benzals*

| Alcohol. | Formula. | Appearance. | Melting point, deg. C. | Solubility. |
|---|---|---|---|---|
| Glycerol-monobenzal. | $C_3H_6O_3 : CHC_6H_5$ | Fine white needles. | 66 | Sol. hot water. |
| Erythrite-dibenzal. | $C_4H_6O_4 (: CHC_6H_5)_2$ | Fine white needles. | 197–8 | Insol. in water. |
| Arabite-monobenzal. | $C_5H_{10}O_5 : CHC_6H_5$ | Fine white needles. | 150 | Sol. hot water. |
| Xylite-dibenzal. | $C_5H_8O_5 (: CHC_6H_5)_2$ | Gelatinous flakes. | 175 | Insol. in water. |
| Adonite-dibenzal. | $C_5H_8O_5 (: CHC_6H_5)_2$ | Fine white needles. | 164–5 | Insol. in water. |
| Mannite-tribenzal. | $C_6H_8O_6 (: CHC_6H_5)_3$ | Fine white needles. | 220 | Insol. in water. |
| Talite-tribenzal. | $C_6H_8O_6 (: CHC_6H_5)_3$ | Fine white needles. | 206 | Insol. in water. |
| Sorbite-dibenzal. | $C_6H_{10}O_6 (: CHC_6H_5)_2$ | $\alpha$ Amorphous. | 200 | Slightly sol. in water. |
| | | $\beta$ Crystalline. | 164 | Insol. in water. |
| Dulcite-dibenzal. | $C_6H_{10}O_6 (: CHC_6H_5)_2$ | Fine white needles. | 215–20 | Sol. hot water. |
| Perseite-dibenzal. | $C_7H_{12}O_7 (: CHC_6H_5)_2$ | Fine white needles. | 230–5 | Insol. in water. |
| Glucoheptite-monobenzal. | $C_7H_{14}O_7 : CHC_6H_5$ | Fine white needles. | 214 | Insol. in water. |

**Oxidation of Sugar Alcohols.** — As the sugars upon reduction yield alcohol derivatives so the latter upon gentle oxidation are converted into sugars. The two processes are not however strictly reversible for while glucose upon reduction gives the alcohol sorbite, the latter upon oxidation gives a mixture of glucose and sorbose. In fact it may be stated as a general rule that the sugar alcohols upon weak oxidation yield both an aldose and a ketose sugar. This may be seen from the following examples:

| Alcohol | | Sugars derived by oxidation. Aldose | | Ketose |
|---|---|---|---|---|
| Glycerol | = | d,l-Glycerose | + | Dioxyacetone |
| i-Erythrite | = | d,l-Erythrose | + | d,l-Erythrulose |
| d-Mannite | = | d-Mannose | + | d-Fructose |
| d-Sorbite | = | d-Glucose | + | d-Sorbose |

*Oxidation of Sugar Alcohols by Chemical Means.* — One method of oxidation frequently used by Fischer * is to treat the alcohol with 10

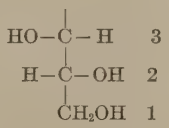
* "Untersuchungen über Kohlenhydrate" (1909), pp. 244, 294, etc.

parts nitric acid of 1.18 sp. gr. at a temperature of about 45° C. The liquid soon acquires reducing properties and when this has reached its maximum the solution is cooled, neutralized, and the sugar precipitated as hydrazone, osazone, or examined by other suitable methods. In place of nitric acid other agents may be used for oxidizing the sugar alcohols to sugars, such, for example, as sodium hypobromite, weak permanganate, hydrogen peroxide in presence of ferrous sulphate, and lead peroxide with hydrochloric acid.

*Oxidation of Sugar Alcohols by Means of Bacteria.* — The oxidation of the sugar alcohols to sugars may also be accomplished by biochemical means. The organism most used for this purpose is the *Bacterium xylinum*, or sorbose bacterium, the action of which upon sugar alcohols and sugars has been especially studied by Bertrand.* The peculiarity of the oxidation of sugar alcohols by *Bacterium xylinum* is that the sugars formed are largely if not entirely ketoses. The following examples are given of oxidation of sugar alcohols made by means of this organism:

Glycerol = Dioxyacetone.
i-Erythrite = Erythrulose.
l-Arabite = Araboketose.
d-Mannite = d-Fructose.
d-Sorbite = d-Sorbose.
Perseite = Heptoketose.
Volemite = Heptoketose.

All the sugar alcohols, however, are not oxidized by *Bacterium xylinum*. Dulcite and xylite, for example, are not affected by this organism. A curious fact noted in this connection is that oxidation by *Bacterium xylinum* does not take place in compounds where the hydroxyl groups in the second and third position lie on opposite sides of the carbon chain. Thus xylite and dulcite both have the following configuration in common:

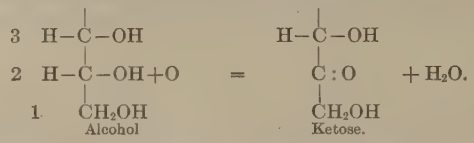



For some reason not understood sugar alcohols having the above arrangement, are not oxidized by *Bacterium xylinum*.

Sorbite, mannite, arabite and erythrite, on the other hand, have the

* Compt. rend., **126,** 762, 894, 984; **130,** 1330; Bull. soc. chim. [3], **15,** 627; **19,** 347.

hydroxyl groups in the second and third position on the same side of the carbon chain and are oxidized by *Bacterium xylinum* as follows:




Too prolonged or too violent oxidation of the sugar alcohols will lead beyond the sugars to the formation of sugar acids, the description of which will follow.

## The Sugar Acids

The sugar acids according to the degree of oxidation are divided into two groups: the monobasic and the dibasic acids.

### THE MONOBASIC ACIDS OF THE SUGARS

**Synthesis of the Monobasic Acids.** — The oxidation of sugars to the monobasic acids is usually accomplished by means of bromine water. The general equation for the reaction with an aldose sugar is:

$$\underset{\text{Aldose}}{C_nH_{2n}O_n} + \underset{\text{Bromine}}{2\,Br} + H_2O = \underset{\text{Aldonic acid}}{C_nH_{2n}O_{n+1}} + \underset{\text{Hydrobromic acid.}}{2\,HBr.}$$

In carrying out the reaction according to Fischer * 1 part of sugar is dissolved in 5 parts of water and 2 parts of bromine added. The solution is kept cold and shaken frequently until all bromine has dissolved. After standing at room temperature 1 to 3 days, the solution is heated to expel any excess of bromine; carbonate of lead is then added to neutralize the hydrobromic acid formed in the reaction and the filtered solution evaporated to about half its volume. After 24 hours the lead bromide, which has crystallized, is filtered off and the lead remaining in solution precipitated with hydrogen sulphide. After boiling off the hydrogen sulphide from the filtrate the last traces of hydrobromic acid are removed from the solution by shaking with silver oxide, and any dissolved silver removed from the filtrate with hydrogen sulphide. The solution is then reboiled to expel hydrogen sulphide, decolorized if necessary with animal charcoal and filtered when the acid can either be precipitated, in the form of an insoluble salt or other derivative, or separated as a crystalline lactone by evaporation.

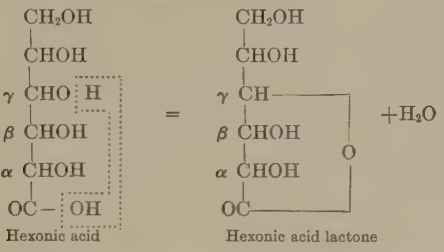
The method just described for oxidizing sugars to their monobasic acids holds true, however, only for the aldose sugars. Ketose sugars are but little affected by bromine water at ordinary temperature during the

* Ber., **22**, 3218.

first few days. Several weeks' contact, however, will bring about slow oxidation, with a breaking up of the molecule into a mixture of acids of lower carbon content.

*Nomenclature.* — In the nomenclature of the monobasic acids derived from the sugars the ending *-onic* is usually substituted for the termination *-ose* of the sugar as xylonic, gluconic, pentonic, hexonic, etc.

**Lactones of the Monobasic Acids.** — Of the monobasic acids glycollic acid, corresponding to a diose sugar, is obtained in the form of crystals (melting point 80° C.) and glyceric acid, corresponding to a triose sugar, as a thick sirup. The higher tetronic, pentonic, hexonic, heptonic, octonic and nononic acids split off one molecule of water upon evaporation and crystallize out as lactones. The formation of the lactone of a hexonic acid is represented as follows:




In the above reaction the splitting off of water and the linkage by the oxygen ring always take place between the carbon atom of the terminal COOH group and the third or γ carbon atom. Glycollic and glyceric acids, which have no γ carbon atom, are unable to form lactones.

The lactones of the monobasic acids are well-defined crystalline compounds easily soluble in water. Freshly prepared aqueous solutions of the lactones are neutral in reaction, but on standing a strong acidity develops owing to the regeneration of the carboxyl group by addition of water.

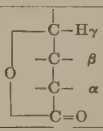
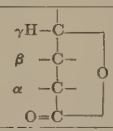
The sugar monobasic acids and their lactones are optically active. A marked difference, however, is noticeable between the specific rotations of the acid and its lactone, and with the transformation of the one into the other, by the addition or splitting off of water, changes in rotation take place which resemble the mutarotation of sugars. This is seen from the following observations made upon galactonic acid and its lactone; to reduce the influence of lactone formation the observations for the free acid were made upon a solution prepared by decomposing a

solution of the calcium salt with the equivalent amount of oxalic acid and filtering.

| | | $[\alpha]_D$. |
|---|---|---|
| Galactonic acid * from calcium salt | 10 minutes after solution | − 10.56 |
| | 5 hours after solution | − 13.77 |
| | 6 days after solution | − 39.24 |
| | 15 days after solution | − 45.90 |
| | 3 weeks after solution | − 46.82 |
| Galactonic acid † lactone | immediately after solution | − 77.61 |
| | several days after solution | − 67.89 |

The observations show a slow conversion of the acid into the lactone and a similar conversion of the lactone into the acid; after a longer or shorter period of time, depending upon temperature and concentration, a condition of equilibrium is reached when the rotation remains constant.

**Relation of Configuration to the Rotation of Lactones.** — An important relation, noted by Hudson,‡ between the configuration and rotation of the lactones of the sugar acids, is that all dextrorotatory lactones have their ring linkages upon one side of the carbon chain and all levorotatory lactones upon the opposite side. In the following table the position of the lactone ring, with reference to the terminal CO group and the carbon chain, is indicated at the head of the two classes of lactones.

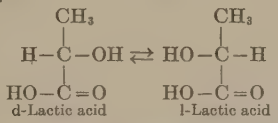
| Dextrorotatory lactones. | | Levorotatory lactones. | |
|---|---|---|---|
|  | |  | |
| l-Xylonic | + 83 | l-Arabonic | − 73.9 |
| d-Lyxonic | + 82.4 | l-Ribonic | − 18.0 |
| d-Gluconic | + 68.2 | Rhamnonic | − 39.0 |
| d-Mannonic | + 53.8 | Isorhamnonic | − 62.0 |
| d-Gulonic | + 56.1 | Rhodeonic | − 76.3 |
| α-Rhamnohexonic | + 83.8 | d-Galactonic | − 77.6 |
| β-Rhamnohexonic | + 43.3 | α-Glucoheptonic | − 55.3 |
| α-Rhamnoheptonic | + 55.6 | β-Glucoheptonic | − 67.7 |
| α-Glucooctonic | + 45.9 | d-Mannoheptonic | − 74.2 |
| β-Glucooctonic | + 23.6 | α-Galaheptonic | − 52.3 |
| α-Galaoctonic | + 64.0 | Rhamnooctonic | − 50.8 |
| | | d-Mannooctonic | − 43.6 |

Anderson § has extended the above list and shows that the relationship discovered by Hudson also holds for the lactones of the

* Schnelle and Tollens, Ber., **23,** 2991.
† Ruff and Franz, Ber., **35,** 948.
‡ J. Am. Chem. Soc., **32,** 338.
§ J. Am. Chem. Soc., **34,** 51.

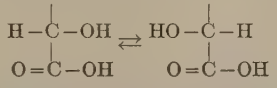
different saccharinic acids, as saccharin, isosaccharin, metasaccharin, etc., (see pp. 587 and 604). The relationship is an important one, since the rotation of a lactone indicates the position of the ring, thus establishing the configuration for the $\gamma$ position of the acid and hence also for the corresponding sugar. In this manner Hudson has not only verified the configurations of the sugars established by Fischer from purely chemical data, but has pointed out the probable structure of a number of sugars whose configurations have been in doubt.

**Molecular Rearrangement of the Sugar Acids.** — A peculiarity of many sugar acids is the ease with which they undergo molecular change into other isomers when their solutions are heated at high temperature. The simplest instance of such a change is the transformation of dextro-lactic into levo-lactic acid or vice versa, the reaction as in all such cases being a reversible one.




The lactones do not appear susceptible to this kind of molecular rearrangement and to prevent their formation the experiment with acids, which yield lactones, is carried out * by heating the aqueous solution at 130° to 150° C. in presence of pyridine or quinoline, the latter through formation of salts preventing the generation of lactones.

The part of the molecule which is affected in this method of isomerization is always the hydroxyl adjoining the carboxyl group, the general formula for the reaction being:



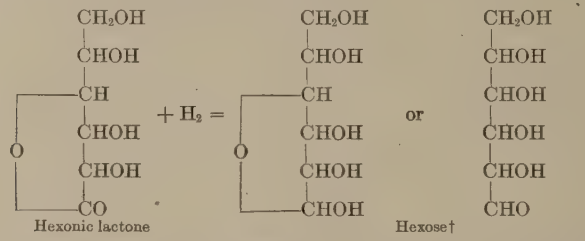

The following examples are given of monobasic acids which have been found to undergo mutual isomerization by the method of Fischer just described.

l-Xylonic ⇄ d-Lyxonic
l-Arabonic ⇄ l-Ribonic
d-Gluconic ⇄ d-Mannonic
l-Gluconic ⇄ l-Mannonic
d-Galactonic ⇄ d-Talonic
l-Gulonic ⇄ l-Idonic

The same reaction is also obtained between the $\alpha$ and $\beta$ isomers of the heptonic, octonic and nononic acids.

* Fischer, Ber., **27**, 3189.

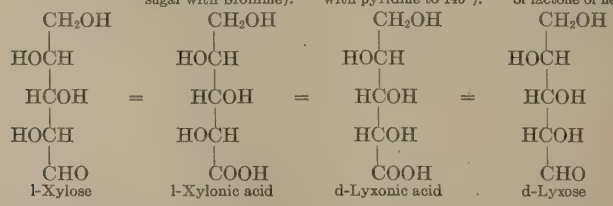
**Reduction of Lactones to Sugars.** — It has not been found possible to reduce the monobasic acids in aqueous solution; the *lactones*,* however, are easily reduced in aqueous solution by means of sodium amalgam first to sugars and after prolonged reduction to sugar alcohols. The reaction for a hexonic lactone would be:




Hexonic lactone — Hexose†

The reaction, according to Fischer,‡ is carried out by treating an ice-cold solution of the lactone in 10 parts of water with sodium amalgam ($2\frac{1}{2}$ per cent sodium), the mixture being always kept weakly acid with sulphuric acid. The reaction is stopped when the reducing power upon Fehling's solution has reached its maximum (usually 30 to 40 minutes). The solution is then neutralized, decolorized with bone black and evaporated to crystallization of sodium sulphate, when it is poured into 20 times its volume of hot alcohol. After cooling, the alcoholic solution is filtered from sodium sulphate and evaporated to a sirup from which the sugar may be separated as hydrazone or other compound according to conditions. The yield of sugar is 40 to 60 per cent of the pure lactone.

*Employment of Method in the Synthesis of New Sugars.* — The transformation of the monobasic acids of known sugars into new isomers and the reduction of the lactones of the new acid by the process just described have been used by Fischer with great success in the synthesis of many new sugars. The following is given as an illustration of the method:




l-Xylose — l-Xylonic acid — d-Lyxonic acid — d-Lyxose

* Fischer, Ber., **22,** 2204.

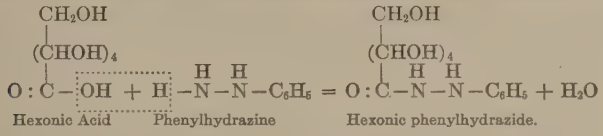
† The sugars are regarded by many chemists as having a lactonic structure similar to the form shown in the equation. The fact that only lactones are reduced to sugars tends to support this view.

‡ Ber., **23,** 930.

In the same manner:

| | | | |
|---|---|---|---|
| l-Arabinose | = l-arabonic acid | = l-ribonic acid | = l-ribose. |
| Rhamnose | = rhamnonic acid | = isorhamnonic acid | = isorhamnose |
| d-Galactose | = d-galactonic acid | = d-talonic acid | = d-talose. |
| d-Gulose | = d-gulonic acid | = d-idonic acid | = d-idose. |
| $\alpha$-Heptose | = $\alpha$-heptonic acid | = $\beta$-heptonic acid | = $\beta$-heptose. |
| $\alpha$-Octose | = $\alpha$-octonic acid | = $\beta$-octonic acid | = $\beta$-octose. |

**Hydrazide* Reaction of the Monobasic Acids.** — Among the most important derivatives of the sugar acids, for purposes of identification and separation, are the phenylhydrazides. All of the acids derived from the sugars react with phenylhydrazine; the resulting product, however, is entirely different in chemical properties from the hydrazones and osazones of the sugars, resembling more the acid amides. The reaction of a hexonic acid with phenylhydrazine is given as illustration:




The reaction is carried out by heating a solution containing 1 part of the acid in 10 parts of water with 1 part of phenylhydrazine and 1 part of 50 per cent acetic acid for three-quarters of an hour upon the water bath. The solution is cooled, the precipitate of phenylhydrazide filtered off, washed with a little cold water and recrystallized from hot water using a little animal black. The hydrazides thus obtained are colorless crystalline compounds, the melting points of which will serve in many cases for purpose of identification.

The phenylhydrazides are decomposed upon heating with alkaline hydroxides, with formation of a salt of the acid and free phenylhydrazine. Barium hydroxide is generally used for this purpose: 1 part of hydrazide is treated with 30 parts of hot 10 per cent barium hydroxide solution, boiled one-half hour and then cooled. The free phenylhydrazine is then extracted with ether, the barium precipitated with the exact amount of sulphuric acid and the solution filtered; the filtrate upon evaporation will yield the lactone of the acid.

**Salts of the Monobasic Acids.** — The monobasic acid derivatives of the sugars give a large number of salts with different metals, some of which have been used for purposes of identification. Mention has been made of a few of these, in so far as they pertain to the identification of sugars, under the reactions of the individual sugars.

* Fischer and Passmore, Ber., **22,** 2728.

The salts of calcium, barium, cadmium, and lead have been employed in some cases for isolating certain of the acids. The cadmium and lead salts (the latter usually amorphous flocculent precipitates) are decomposed after separation with hydrogen sulphide and the calcium and barium salts with the equivalent amounts of oxalic or sulphuric acid; the precipitates are filtered off and the liberated acid is obtained by concentrating the filtrate.

A number of the monobasic acids give characteristic salts with different alkaloids, as strychnine, brucine, morphine, and the various cinchona bases. The utilization of these salts in analyzing racemic mixtures of sugar acids will be described later (p. 786).

**Oxidation of Monobasic Acids of the Sugars.** — The monobasic acid derivatives of the unsubstituted aldose sugars are converted by oxidizing agents (as nitric acid, 1.2 sp. gr.) into the corresponding dibasic acids; the substituted monobasic acids, rhamnonic, fuconic, rhodeonic, methylhexonic, etc., yield dibasic acids of one less carbon atom with loss of the methyl group.

### THE DIBASIC ACIDS OF THE SUGARS

**Formation.** — The oxidation of sugars to their dibasic acids is usually performed by warming the sugar with 30 per cent nitric acid. The reaction only holds for normal unsubstituted aldose sugars, the ketoses being all degraded into lower oxidation products, of which oxalic acid is usually formed in largest amount. The oxidation of an aldohexose sugar to its dibasic acid by means of nitric acid proceeds as follows:

$$\begin{array}{l} CH_2OH \\ (CHOH)_4 \\ H-C:O \end{array} + 2\,HNO_3 = \begin{array}{l} O:C-OH \\ (CHOH)_4 \\ O:C-OH \end{array} + 2\,H_2O + 2\,NO$$

*Nomenclature.* — The nomenclature of the dibasic acids is irregular. In some cases where there is a genetic relationship, as between the sugars glucose, mannose, and idose, and their dibasic acids saccharic, mannosaccharic, and idosaccharic, a certain uniformity exists; so also between the sugars galactose and talose, and their dibasic acids mucic and talomucic. The family to which each acid belongs is usually indicated by the name of the saturated dibasic fatty acid having the same number of carbon atoms, as: malonic (3 C atoms), succinic (4), glutaric (5), adipic (6), pimelic (7), suberic (8) and azelaic (9).

| Sugar. | | Dibasic acid. | |
|---|---|---|---|
| Class. | Formula. | Class. | Formula. |
| Triose | $C_3H_6O_3$ | Oxymalonic* | $C_3H_4O_5$ |
| Tetrose | $C_4H_8O_4$ | Dioxysuccinic | $C_4H_6O_6$ |
| Pentose | $C_5H_{10}O_5$ | Trioxyglutaric | $C_5H_8O_7$ |
| Hexose | $C_6H_{12}O_6$ | Tetroxyadipic | $C_6H_{10}O_8$ |
| Heptose | $C_7H_{14}O_7$ | Pentoxypimelic | $C_7H_{12}O_9$ |
| Octose | $C_8H_{16}O_8$ | Hexoxysuberic | $C_8H_{14}O_{10}$ |
| Nonose | $C_9H_{18}O_9$ | Heptoxyazelaic | $C_9H_{16}O_{11}$ |

**Properties of the Dibasic Acid Derivatives of Sugars.** — The possession of an additional carboxyl group gives the dibasic acids of the sugars certain properties which distinguish them from the monobasic acids. Among these properties may be mentioned, (1) The formation of lactone acids and double lactones; (2) The formation of two classes of hydrazides, the single and double; (3) The formation of several classes of salts, the acid, neutral, and double.

**Lactone Acids.** — The formation of lactones is not so general with the dibasic as with the monobasic acids. With the tetrose derivatives the $\gamma$ position, which is held by an alcohol group in the monobasic acids, is occupied by one of the carboxyl groups in the dibasic acids (d-, l-, and i-tartaric acids) so that lactone formation is excluded. But even in the case of some of the higher derivatives, as of arabinose, xylose, and galactose, the dibasic acid crystallizes out in the free condition. Mucic acid, derived from galactose, can be converted, however, into a monolactone by long boiling with water.

The lactones of the dibasic acids are in nearly all cases mono- or acid lactones: in other words only one of the carboxyl groups is affected, the other remaining free and retaining its acid properties. The monolactone of saccharic acid, for example, can be represented by the formula

* The prefix *oxy-* is loosely used instead of *hydroxy-*. According to the nomenclature of the Geneva Congress, which is but little followed, the dibasic acid of a pentose sugar would be pentane-triol-dicarboxylic acid; of a hexose, hexane-tetrol-dicarboxylic acid; of a heptose, heptane-pentol-dicarboxylic acid, etc.

The lactone acids are nearly all crystalline compounds, easily soluble in water. The solution of a lactone acid, neutralized in the cold with sodium hydroxide, quickly becomes acid again through reconversion of the lactone into the free acid group. Stable compounds of the lactone acids are for this reason unknown.

Solutions of the lactone acids in water undergo spontaneously a partial change into the dibasic acid with establishment of a condition of equilibrium, the predominance of lactone acid, or of dibasic acid, depending upon the temperature and concentration. With this transformation changes are noted in the rotation of the solution. In the case of saccharic acid and its lactone acid, the following specific rotations were noted.

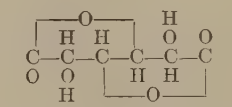
| | $[\alpha]_D$. |
|---|---|
| Saccharic acid,* after solution | + 9.1 |
| Saccharic acid, constant (29 days) | + 22.7 |
| Saccharic acid monolactone, after solution | + 37.9 |
| Saccharic acid monolactone, constant (56 days) | + 22.5 |

The results show that the change between saccharic acid and its lactone is a reversible one, the same condition of equilibrium being reached whichever compound is first dissolved. The case is similar to that of galactonic acid and its lactone (p. 774).

**Double Lactones.** — With the dibasic acids derived from d- and l-mannose, the peculiarity of double lactone† formation is observed. These very characteristic compounds crystallize out with 2 molecules of water, which can be eliminated by drying over concentrated sulphuric acid. Aqueous solutions of the double lactones are at first neutral, but become acid upon standing; the aqueous solutions have also the peculiarity of strongly reducing Fehling's solution, this being probably due to an aldehydic rearrangement of the dilactone molecule in presence of free alkalies.

The rotations of the lactone acids and double lactones agree perfectly with Hudson's hypothesis (p. 774) according to which the character of rotation depends upon the position of the lactone ring.

The structure of the double lactone of d-mannosaccharic acid is shown as follows:

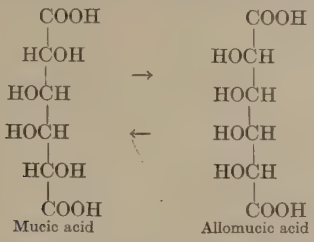


$[\alpha]_D = +200.$

* Tollens and Sohst, Chem. Ztg., **11,** 99, 1178; Ann., **245, 1.**

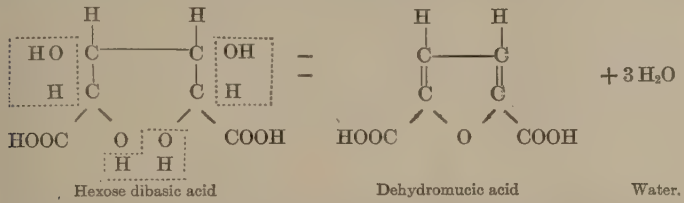
† Kiliani, Ber., **20,** 341; Fischer, Ber., **24,** 539.

The property of undergoing transformation to other isomers upon heating with pyridine at 140°, noted for the monobasic acids (p. 775), also exists with the dibasic acids. Mucic acid has been converted in this way by Fischer * into the isomeric compound allomucic acid.




As with the monobasic acids the HCOH groups adjoining COOH radicals are the parts of the molecule affected in this reaction.

**Dehydration of Dibasic Acids of Hexoses.** — A noteworthy characteristic of the dibasic acids of the hexoses is the ease with which they undergo dehydration, upon heating to 150° C. with concentrated hydrochloric acid, hydrobromic acid, sulphuric acid or other dehydrating agent, with formation of the unsaturated dehydromucic acid. The reaction is illustrated graphically as follows:

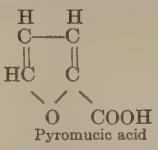


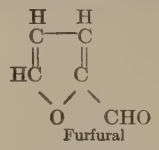

*Dehydromucic Acid.*—The best dehydrating agent to use for the above reaction, according to Fischer,* is a mixture of hydrochloric and hydrobromic acids. Fischer considers the dehydromucic acid reaction the best of all methods for detecting a dibasic acid of the hexose type.

Dehydromucic acid is best recognized by the reaction of Tollens and Yoder †: 2 to 5 mgs. of substance are carefully heated with 2 c.c. concentrated sulphuric acid and 1 to 4 mgs. of isatin at 145° to 155° C. When the test is made with pure dehydromucic acid the solution will be colored a strong violet blue; with the dibasic hexose acids (mucic, saccharic, mannosaccharic, etc.), the solution takes on more of a green color and shows before the spectroscope two characteristic absorption bands near the $\alpha$ and $\beta$ lines of strontium.

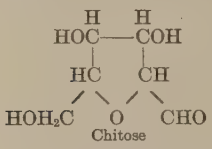
* Ber., **24**, 2136. † Ber., **34**, 3448.

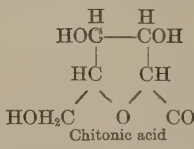
Dehydromucic acid upon heating splits off $CO_2$ and yields pyromucic acid which is the acid derivative of furfural (p. 374).

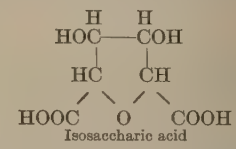

Pyromucic acid


Furfural

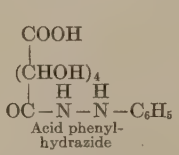
*Chitonic and Isosaccharic Acids.* — Resembling dehydromucic acid in structure are the saturated monobasic and dibasic acids derived from chitose, which is probably also itself a saturated furfuran derivative.

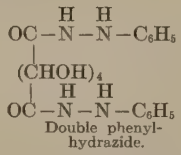

Chitose


Chitonic acid


Isosaccharic acid

**Hydrazides of Dibasic Acids.** — The dibasic acids of the sugars yield hydrazides the same as the monobasic derivatives; the second carboxyl group enables them however to fix an additional molecule of phenylhydrazine. Many of the dibasic acids give, in fact, two classes of compounds, the acid and double hydrazides. The acid hydrazides are precipitated usually with phenylhydrazine in the cold and the double hydrazides by heating. The following formulæ illustrate the configuration of the acid and double hydrazides:


Acid phenyl-hydrazide

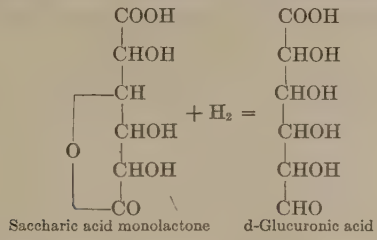

Double phenyl-hydrazide.

The acid hydrazides are colorless compounds easily soluble in hot water, while the double hydrazides are usually of a pale yellow color and only slightly soluble in hot water.

**Reduction of Dibasic Acids.** — The lactones of the dibasic acids are reduced by sodium amalgam, following the same method described on p. 776, and yield in succession the lactones of the monobasic acid, the sugars and the corresponding alcohols.

*d-Glucuronic Acid.* — An interesting intermediary step between the dibasic and monobasic acids, noted in the reduction of the lactones of saccharic and mucic acids, is the production of an aldehyde acid. In

the case of saccharic acid monolactone, for example, Fischer and Piloty* obtained as an intermediary reduction product d-glucuronic acid.




Saccharic acid monolactone — d-Glucuronic acid

d-Glucuronic acid occurs naturally in the urine and yields furfural upon distillation with hydrochloric acid; its properties, reactions, and close relationship to the pentoses are referred to elsewhere (p. 375).

The successive steps in the reduction of different lactones of the dibasic acids are given as follows:

| Dibasic acid lactone. | Aldehyde acid. | Monobasic acid lactone. | Sugar. | Alcohol. |
|---|---|---|---|---|
| Saccharic acid | d-Glucuronic | d-Gulonic | d-Gulose | Sorbite |
| Mannosaccharic acid | ? | d-Mannonic | d-Mannose | Mannite |
| Mucic acid | Galacturonic | d, l-Galactonic | d, l-Galactose | Dulcite |

**Salts of the Dibasic Acids.** — The dibasic acid derivatives of the sugars yield a large variety of salts; the formation of acid and double salts is in general a distinguishing feature of the dibasic as compared with the monobasic acids.

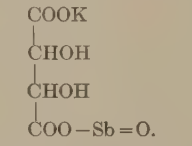
Many of the dibasic acids give insoluble compounds with calcium, lead and other metals, and some of these (as calcium oxalate) are used considerably for purposes of separation and analysis. The calcium salts of the higher dibasic acids can usually be precipitated from cold aqueous solution; after filtering and dissolving in hot water the calcium can be removed by treating with an exactly equivalent quantity of oxalic acid. The calcium oxalate is then filtered off and the pure acid obtained in the filtrate. The isolation of the acids can also be effected by means of the lead salts; the latter after precipitation are filtered off, washed and then decomposed in aqueous suspension with hydrogen sulphide. The lead sulphide is filtered off and the acid obtained in the filtrate.

Of the acid salts of the dibasic acids those of potassium have the greatest importance. Several of these, as the acid potassium tartrate (cream of tartar) and acid potassium saccharate, are characterized by

* Ber., **24**, 521.

low solubility in cold water and this property is made use of in the identification of these acids.

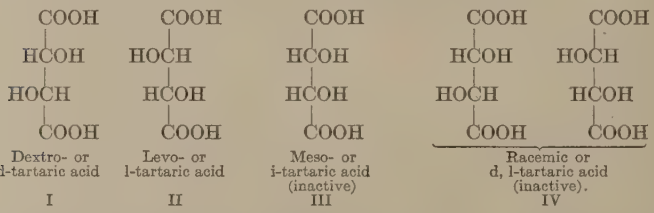
There are a large number of interesting double salts of the dibasic acids but only a few of these can be mentioned. Several dibasic acids, as tartaric, saccharic and mucic, give double compounds with potassium and antimony oxide. Of these potassium antimonyl tartrate, or tartar emetic, is given as illustration:



Of other double salts the sodium ammonium tartrates have a special historical interest, since it was owing to the work of Pasteur upon these salts that the science of molecular asymmetry and the methods for analyzing racemic mixtures had their first beginning. The problem of separating the dextro- and levo-rotatory components of an optically inactive racemic mixture was in fact first solved by Pasteur; as the methods established by him are still the ones most generally employed, this particular branch of sugar analysis may be treated best in connection with a review of Pasteur's work upon tartaric acid.

### The Analysis of Racemic Mixtures

Tartaric acid may be said to exist under four different forms; the structural formulæ of these are represented as follows:




The d- and l-components of a racemic* mixture usually resemble one another in melting point, solubility, specific gravity, chemical affinity, and all other properties except specific rotation; the racemic substance itself may differ, however, from its components in crystalline form, melting point, solubility, and other characteristics. In other words a racemic compound may behave not as a mixture, but as a simple sub-

* The word racemic is derived from the Latin for tartaric acid, *acidum racemicum*, where the phenomenon was first noted.

stance; it is this peculiarity which renders the separation of the two optically active antipodes in a racemic mixture a matter of such difficulty.

In many laboratory operations where optically active substances are formed, the d- and l-isomers are produced in equal amounts; many instances of optical activity escape notice for this very reason. The possibility of separating an optically inactive compound into two optically active components should therefore always be considered.

**Separation of Racemic Mixtures by Differences in Crystalline Form.** — It was observed by Pasteur* in 1848 that when a solution of racemic

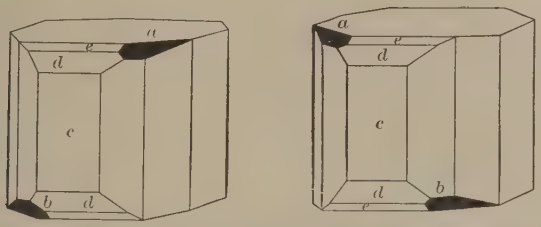



Fig. 200. — Showing opposite hemihedrism of crystals of the sodium-ammonium salts of d-tartaric and l-tartaric acids.

acid which had been neutralized, one-half with sodium hydroxide and one-half with ammonia, was allowed to evaporate under certain conditions, separate crystals were obtained of the d- and l- double salts. The two classes of salts were similar in all respects except in the position of their hemihedral faces (shown in black, Fig. 200). In one set of crystals, for example, the hemihedral faces were always at the right of the surfaces *d* and *e*, when the latter were uppermost, and in the other set of crystals always at the left. The relationship between the two crystalline forms was exactly like that between one crystal and its mirror image, where one form cannot be brought into coincidence with the other by any method of turning the crystal.

By dissolving separately the two sets of hemihedral crystals, Pasteur obtained in one case a solution which rotated the plane of polarized light to the right, and in the other case a solution which rotated the plane of polarized light to the left. Separation was thus effected of

* For a full account of Pasteur's researches upon the tartaric acids see his "Récherches sur la dissymmetrie moléculaire des produits organiques naturels," Paris; also his original papers, Compt. rend., **26,** 535; **27,** 401; **32,** 110; **35,** 180; **36,** 26; **37,** 110, 162; etc.

the inactive racemic salt into its two optically active components. The phenomenon of hemihedrism was explained by Pasteur as due to an asymmetric arrangement of the atoms within the molecule, the grouping in one compound being exactly the reverse of that in the other.

If the d, l-sodium ammonium tartrate crystallizes out at a high temperature only the non-hemihedral crystals of the racemate are obtained. The transition point between separation of racemate and that of the hemihedral crystals of d- and l-tartrate is 28° C.; and it is only under this temperature that separation of the two salts can be effected by the difference in crystalline form.

Separations of racemic mixtures into their optically active components by differences in crystalline form have been made upon other sugar derivatives. The optically inactive lactone of d, l-gulonic acid, for example, crystallizes, according to Haushofer,* in rhombic crystals with hemihedral faces; by selecting the forms of opposite hemihedry the d- and l-lactones are obtained of opposite specific rotation. This means of separation is not, however, generally applicable, and recourse is usually made to other methods.

**Separation of Racemic Mixtures by Combination with Other Optically Active Compounds.** — This second method of separating racemic mixtures is also due to Pasteur, who discovered that when a hot aqueous solution of d, l-tartaric acid was saturated with equivalent amounts of different cinchona bases the quinine and quinicine salts of d-tartaric acid crystallized out before the corresponding compounds of l-tartaric acid, while the cinchonine and cinchonicine salts of l-tartaric acid separated before the corresponding compounds of d-tartaric acid. This method of separating racemic mixtures has been greatly extended since the time of Pasteur and has been applied to many different classes of compounds. In many operations where sugar acids are formed, both optical antipodes are produced, the inactive racemic mixture of the d- and l-acids behaving very much as a simple acid and yielding upon evaporation an optically inactive lactone.

The salts of the alkaloids have been of great service in separating the d- and l-components of different inactive sugar acids. The strychnine salt of d-mannonic acid,† for example, is soluble in hot absolute alcohol, while the strychnine salt of l-mannonic acid is insoluble. If the latter is filtered off, dissolved in water and treated with barium hydroxide solution, the strychnine is precipitated and a soluble barium salt of l-mannonic acid formed. The solution is filtered, shaken out with ether to

* Ber., **24,** 530; **25,** 1027.

† Fischer, Ber., **23,** 370.

remove any remaining strychnine and then treated with sulphuric acid in exact amount to precipitate all barium sulphate. The latter is filtered off and the filtrate evaporated when the l-mannonic acid will crystallize out as a lactone. In the same manner d-galactonic acid (strychnine salt of low solubility) has been separated from l-galactonic acid.

The principal alkaloids used for separating racemic mixtures of acids are the cinchona bases, quinine, quinidine, cinchonine and cinchonidine; the strychnos bases, strychnine and brucine; and the opium base, morphine.

The principle of this method has also been employed in separating racemic mixtures of sugars by means of optically active hydrazines (p. 361).

**Separation of Racemic Mixtures by Selective Fermentation.**

This third method of separating racemic mixtures is also due to Pasteur and is based upon the difference in susceptibility of the d- and l-components to attack by different ferments and moulds. Pasteur noted that inactive solutions of ammonia d, l-tartrate after inoculation with spores of *Penicillium glaucum* (in presence of slight amounts of mineral salts to act as nutrients) became strongly levorotatory. This was explained by the fact that the d-tartaric acid was fermented by the mould, the l-tartaric acid remaining unaffected.

Pasteur's third method of resolving racemic compounds has also been greatly extended and has been employed with success in separating mixtures of d, l-sugars and acids. Thus by means of yeast Fischer was able to ferment the d-sugar in d, l-glucose, d, l-mannose, d, l-galactose and d, l-fructose, and obtain the l-sugar in a pure condition.

For separating the sugar acids, *Penicillium glaucum*, first used by Pasteur, is still largely employed. Of the acids fermented by this mould may be mentioned d-tartaric, d-glyceric, d-mannonic, and d-glutaminic acids, the l-isomers of these compounds not being attacked. The selective influence of a mould, yeast, or other organism is not confined, however, to the members of a single d- or l-series as might be inferred from the examples mentioned. Thus with the ammonium salt of d, l-lactic acid (fermentation lactic acid), the l-lactic acid is fermented by *Penicillium glaucum* and the d-compound left behind in solution.

# APPENDIX OF SUGAR TABLES

# INTRODUCTION

The following tables, which have been selected to accompany various methods described in the author's "Handbook of Sugar Analysis," have been grouped together for convenience as a separate Appendix. This arrangement was made partly to prevent breaking the continuity of the text by the introduction of lengthy tables and partly to permit the publication of the Appendix as a separate book for the convenience of those who have occasion to make use of special tables in the laboratory.

Knowing the very diverse preferences of individual sugar chemists, the author has made a rather wide selection from the more commonly used copper reduction tables. Limitations of space have obliged him, however, to leave out many tables of recognized merit and this must be his excuse for any errors of omission.

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## TABLE * 1.

SPECIFIC GRAVITY OF SUCROSE SOLUTIONS AT $\frac{20°}{4°}$ C. (KAISERLICHE NORMAL-EICHUNGS-KOMMISSION.)

| Per cent sucrose | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.998234 | 0.998622 | 0.999010 | 0.999398 | 0.999786 | 1.000174 | 1.000563 | 1.000952 | 1.001342 | 1.001731 |
| 1 | 1.002120 | 1.002509 | 1.002897 | 1.003286 | 1.003675 | 1.004064 | 1.004453 | 1.004844 | 1.005234 | 1.005624 |
| 2 | 1.006015 | 1.006405 | 1.006796 | 1.007188 | 1.007580 | 1.007972 | 1.008363 | 1.008755 | 1.009148 | 1.009541 |
| 3 | 1.009934 | 1.010327 | 1.010721 | 1.011115 | 1.011510 | 1.011904 | 1.012298 | 1.012694 | 1.013089 | 1.013485 |
| 4 | 1.013881 | 1.014277 | 1.014673 | 1.015070 | 1.015467 | 1.015864 | 1.016261 | 1.016659 | 1.017058 | 1.017456 |
| 5 | 1.017854 | 1.018253 | 1.018652 | 1.019052 | 1.019451 | 1.019851 | 1.020251 | 1.020651 | 1.021053 | 1.021454 |
| 6 | 1.021855 | 1.022257 | 1.022659 | 1.023061 | 1.023463 | 1.023867 | 1.024270 | 1.024673 | 1.025077 | 1.025481 |
| 7 | 1.025885 | 1.026289 | 1.026694 | 1.027099 | 1.027504 | 1.027910 | 1.028316 | 1.028722 | 1.029128 | 1.029535 |
| 8 | 1.029942 | 1.030349 | 1.030757 | 1.031165 | 1.031573 | 1.031982 | 1.032391 | 1.032800 | 1.033209 | 1.033619 |
| 9 | 1.034029 | 1.034439 | 1.034850 | 1.035260 | 1.035671 | 1.036082 | 1.036494 | 1.036906 | 1.037318 | 1.037730 |
| 10 | 1.038143 | 1.038556 | 1.038970 | 1.039383 | 1.039797 | 1.040212 | 1.040626 | 1.041041 | 1.041456 | 1.041872 |
| 11 | 1.042288 | 1.042704 | 1.043121 | 1.043537 | 1.043954 | 1.044370 | 1.044788 | 1.045206 | 1.045625 | 1.046043 |
| 12 | 1.046462 | 1.046881 | 1.047300 | 1.047720 | 1.048140 | 1.048559 | 1.048980 | 1.049401 | 1.049822 | 1.050243 |
| 13 | 1.050665 | 1.051087 | 1.051510 | 1.051933 | 1.052356 | 1.052778 | 1.053202 | 1.053626 | 1.054050 | 1.054475 |
| 14 | 1.054900 | 1.055325 | 1.055751 | 1.056176 | 1.056602 | 1.057029 | 1.057455 | 1.057882 | 1.058310 | 1.058737 |
| 15 | 1.059165 | 1.059593 | 1.060022 | 1.060451 | 1.060880 | 1.061308 | 1.061738 | 1.062168 | 1.062598 | 1.063029 |
| 16 | 1.063460 | 1.063892 | 1.064324 | 1.064756 | 1.065188 | 1.065621 | 1.066054 | 1.066487 | 1.066921 | 1.067355 |
| 17 | 1.067789 | 1.068223 | 1.068658 | 1.069093 | 1.069529 | 1.069964 | 1.070400 | 1.070836 | 1.071273 | 1.071710 |
| 18 | 1.072147 | 1.072585 | 1.073023 | 1.073461 | 1.073900 | 1.074338 | 1.074777 | 1.075217 | 1.075657 | 1.076097 |
| 19 | 1.076537 | 1.076978 | 1.077419 | 1.077860 | 1.078302 | 1.078744 | 1.079187 | 1.079629 | 1.080072 | 1.080515 |
| 20 | 1.080959 | 1.081403 | 1.081848 | 1.082292 | 1.082737 | 1.083182 | 1.083628 | 1.084074 | 1.084520 | 1.084967 |
| 21 | 1.085414 | 1.085861 | 1.086309 | 1.086757 | 1.087205 | 1.087652 | 1.088101 | 1.088550 | 1.089000 | 1.089450 |
| 22 | 1.089900 | 1.090351 | 1.090802 | 1.091253 | 1.091704 | 1.092155 | 1.092607 | 1.093060 | 1.093513 | 1.093966 |
| 23 | 1.094420 | 1.094874 | 1.095328 | 1.095782 | 1.096236 | 1.096691 | 1.097147 | 1.097603 | 1.098058 | 1.098514 |
| 24 | 1.098971 | 1.099428 | 1.099886 | 1.100344 | 1.100802 | 1.101259 | 1.101718 | 1.102177 | 1.102637 | 1.103097 |

* *See* "Handbook," page 30.

TABLE 1. *(Continued.)*

| Per cent sucrose. | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 1.103557 | 1.104017 | 1.104478 | 1.104938 | 1.105400 | 1.105862 | 1.106324 | 1.106786 | 1.107248 | 1.107711 |
| 26 | 1.108175 | 1.108639 | 1.109103 | 1.109568 | 1.110033 | 1.110497 | 1.110963 | 1.111429 | 1.111895 | 1.112361 |
| 27 | 1.112828 | 1.113295 | 1.113763 | 1.114229 | 1.114697 | 1.115166 | 1.115635 | 1.116104 | 1.116572 | 1.117042 |
| 28 | 1.117512 | 1.117982 | 1.118453 | 1.118923 | 1.119395 | 1.119867 | 1.120339 | 1.120812 | 1.121284 | 1.121757 |
| 29 | 1.122231 | 1.122705 | 1.123179 | 1.123653 | 1.124128 | 1.124603 | 1.125079 | 1.125555 | 1.126030 | 1.126507 |
| 30 | 1.126984 | 1.127461 | 1.127939 | 1.128417 | 1 128896 | 1.129374 | 1.129853 | 1.130332 | 1.130812 | 1.131292 |
| 31 | 1.131773 | 1.132254 | 1.132735 | 1.133216 | 1.133698 | 1.134180 | 1.134663 | 1.135146 | 1.135628 | 1.136112 |
| 32 | 1.136596 | 1.137080 | 1.137565 | 1.138049 | 1.138534 | 1.139020 | 1.139506 | 1.139993 | 1.140479 | 1.140966 |
| 33 | 1.141453 | 1.141941 | 1.142429 | 1.142916 | 1.143405 | 1.143894 | 1.144384 | 1.144874 | 1.145363 | 1.145854 |
| 34 | 1.146345 | 1.146836 | 1.147328 | 1.147820 | 1.148313 | 1.148805 | 1.149298 | 1.149792 | 1.150286 | 1.150780 |
| 35 | 1.151275 | 1.151770 | 1.152265 | 1.152760 | 1.153256 | 1.153752 | 1.154249 | 1.154746 | 1.155242 | 1.155740 |
| 36 | 1.156238 | 1.156736 | 1.157235 | 1.157733 | 1.158233 | 1.158733 | 1.159233 | 1.159733 | 1.160233 | 1.160734 |
| 37 | 1.161236 | 1.161738 | 1.162240 | 1.162742 | 1.163245 | 1.163748 | 1.164252 | 1.164756 | 1.165259 | 1.165764 |
| 38 | 1.166269 | 1.166775 | 1.167281 | 1.167786 | 1.168293 | 1.168800 | 1.169307 | 1.169815 | 1.170322 | 1.170831 |
| 39 | 1.171340 | 1.171849 | 1.172359 | 1.172869 | 1.173379 | 1.173889 | 1.174400 | 1.174911 | 1.175423 | 1.175935 |
| 40 | 1.176447 | 1.176960 | 1.177473 | 1.177987 | 1.178501 | 1.179014 | 1.179527 | 1.180044 | 1.180560 | 1.181076 |
| 41 | 1.181592 | 1.182108 | 1.182625 | 1.183142 | 1.183660 | 1.184178 | 1.184696 | 1.185215 | 1.185734 | 1.186253 |
| 42 | 1.186773 | 1.187293 | 1.187814 | 1 188335 | 1.188856 | 1.189379 | 1.189901 | 1.190423 | 1.190946 | 1.191469 |
| 43 | 1.191993 | 1.192517 | 1.193041 | 1.193565 | 1.194090 | 1.194616 | 1.195141 | 1.195667 | 1.196193 | 1.196720 |
| 44 | 1.197247 | 1.197775 | 1.198303 | 1.198832 | 1.199360 | 1.199890 | 1.200420 | 1.200950 | 1.201480 | 1.202010 |
| 45 | 1.202540 | 1.203071 | 1.203603 | 1.204136 | 1.204668 | 1.205200 | 1.205733 | 1.206266 | 1.206801 | 1.207335 |
| 46 | 1.207870 | 1.208405 | 1.208940 | 1.209477 | 1.210013 | 1.210549 | 1.211086 | 1.211623 | 1.212162 | 1.212700 |
| 47 | 1.213238 | 1.213777 | 1.214317 | 1.214856 | 1.215395 | 1.215936 | 1.216476 | 1.217017 | 1.217559 | 1.218101 |
| 48 | 1.218643 | 1.219185 | 1.219729 | 1.220272 | 1.220815 | 1.221360 | 1.221904 | 1.222449 | 1.222995 | 1.223540 |
| 49 | 1.224086 | 1.224632 | 1.225180 | 1.225727 | 1.226274 | 1.226823 | 1.227371 | 1.227919 | 1.228469 | 1.229018 |

TABLE 1. *(Continued.)*

| Per cent sucrose. | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 1.229567 | 1.230117 | 1.230668 | 1.231219 | 1.231770 | 1.232322 | 1.232874 | 1.233426 | 1.233979 | 1.234532 |
| 51 | 1.235085 | 1.235639 | 1.236194 | 1.236748 | 1.237303 | 1.237859 | 1.238414 | 1.238970 | 1.239527 | 1.240084 |
| 52 | 1.240641 | 1.241198 | 1.241757 | 1.242315 | 1.242873 | 1.243433 | 1.243992 | 1.244552 | 1.245113 | 1.245673 |
| 53 | 1.246234 | 1.246795 | 1.247358 | 1.247920 | 1.248482 | 1.249046 | 1.249609 | 1.250172 | 1.250737 | 1.251301 |
| 54 | 1.251866 | 1.252431 | 1.252997 | 1.253563 | 1.254129 | 1.254697 | 1.255264 | 1.255831 | 1.256400 | 1.256967 |
| 55 | 1.257535 | 1.258104 | 1.258674 | 1.259244 | 1.259815 | 1.260385 | 1.260955 | 1.261527 | 1.262099 | 1.262671 |
| 56 | 1.263243 | 1.263816 | 1.264390 | 1.264963 | 1.265537 | 1.266112 | 1.266686 | 1.267261 | 1.267837 | 1.268413 |
| 57 | 1.268989 | 1.269565 | 1.270143 | 1.270720 | 1.271299 | 1.271877 | 1.272455 | 1.273035 | 1.273614 | 1.274194 |
| 58 | 1.274774 | 1.275354 | 1.275936 | 1.276517 | 1.277098 | 1.277680 | 1.278262 | 1.278844 | 1.279428 | 1.280011 |
| 59 | 1.280595 | 1.281179 | 1.281764 | 1.282349 | 1.282935 | 1.283521 | 1.284107 | 1.284694 | 1.285281 | 1.285869 |
| 60 | 1.286456 | 1.287044 | 1.287633 | 1.288222 | 1.288811 | 1.289401 | 1.289991 | 1.290581 | 1.291172 | 1.291763 |
| 61 | 1.292354 | 1.292946 | 1.293539 | 1.294131 | 1.294725 | 1.295318 | 1.295911 | 1.296506 | 1.297100 | 1.297696 |
| 62 | 1.298291 | 1.298886 | 1.299483 | 1.300079 | 1.300677 | 1.301274 | 1.301871 | 1.302470 | 1.303068 | 1.303668 |
| 63 | 1.304267 | 1.304867 | 1.305467 | 1.306068 | 1.306669 | 1.307271 | 1.307872 | 1.308475 | 1.309077 | 1.309680 |
| 64 | 1.310282 | 1.310885 | 1.311489 | 1.312093 | 1.312699 | 1.313304 | 1.313909 | 1.314515 | 1.315121 | 1.315728 |
| 65 | 1.316334 | 1.316941 | 1.317549 | 1.388157 | 1.318766 | 1.319374 | 1.319983 | 1.320593 | 1.321203 | 1.321814 |
| 66 | 1.322425 | 1.323036 | 1.323648 | 1.324259 | 1.324872 | 1.325484 | 1.326097 | 1.326711 | 1.327325 | 1.327940 |
| 67 | 1.328554 | 1.329170 | 1.329785 | 1.330401 | 1.331017 | 1.331633 | 1.332250 | 1.332868 | 1.333485 | 1.334103 |
| 68 | 1.334722 | 1.335342 | 1.335961 | 1.336581 | 1.337200 | 1.337821 | 1.338441 | 1.339063 | 1.339684 | 1.340306 |
| 69 | 1.340928 | 1.341551 | 1.342174 | 1.342798 | 1.343421 | 1.344046 | 1.344671 | 1.345296 | 1.345922 | 1.346547 |
| 70 | 1.347174 | 1.347801 | 1.348427 | 1.349055 | 1.349682 | 1.350311 | 1.350939 | 1.351568 | 1.352197 | 1.352827 |
| 71 | 1.353456 | 1.354087 | 1.354717 | 1.355349 | 1.355980 | 1.356612 | 1.357245 | 1.357877 | 1.358511 | 1.359144 |
| 72 | 1.359778 | 1.360413 | 1.361047 | 1.361682 | 1.362317 | 1.362953 | 1.363590 | 1.364226 | 1.364864 | 1.365501 |
| 73 | 1.366139 | 1.366777 | 1.367415 | 1.368054 | 1.368693 | 1.369333 | 1.369973 | 1.370613 | 1.371254 | 1.371894 |
| 74 | 1.372536 | 1.373178 | 1.373820 | 1.374463 | 1.375105 | 1.375749 | 1.376392 | 1.377036 | 1.377680 | 1.378326 |

TABLE 1. (*Concluded.*)

| Per cent sucrose. | .0 | .1 | .2 | .3 | .4 | .5 | .6 | .7 | .8 | .9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 75 | 1.378971 | 1.379617 | 1.380262 | 1.380909 | 1.381555 | 1.382203 | 1.382851 | 1.383499 | 1.384148 | 1.384796 |
| 76 | 1.385446 | 1.386096 | 1.386745 | 1.387396 | 1.388045 | 1.388696 | 1.389347 | 1.389999 | 1.390651 | 1.391303 |
| 77 | 1.391956 | 1.392610 | 1.393263 | 1.393917 | 1.394571 | 1.395226 | 1.395881 | 1.396536 | 1.397192 | 1.397848 |
| 78 | 1.398505 | 1.399162 | 1.399819 | 1.400477 | 1.401134 | 1.401793 | 1.402452 | 1.403111 | 1.403771 | 1.404430 |
| 79 | 1.405091 | 1.405752 | 1.406412 | 1.407074 | 1.407735 | 1.408398 | 1.409061 | 1.409723 | 1.410387 | 1.411051 |
| 80 | 1.411715 | 1.412380 | 1.413044 | 1.413709 | 1.414374 | 1.415040 | 1.415706 | 1.416373 | 1.417039 | 1.417707 |
| 81 | 1.418374 | 1.419043 | 1.419711 | 1.420380 | 1.421049 | 1.521719 | 1.422390 | 1.423059 | 1.423730 | 1.424400 |
| 82 | 1.425072 | 1.425744 | 1.426416 | 1.427089 | 1.427761 | 1.428435 | 1.429109 | 1.429782 | 1.430457 | 1.431131 |
| 83 | 1.431807 | 1.432483 | 1.433158 | 1.433835 | 1.434511 | 1.435188 | 1.435866 | 1.436543 | 1.437222 | 1.437900 |
| 84 | 1.438579 | 1.439259 | 1.439938 | 1.440619 | 1.441299 | 1.441980 | 1.442661 | 1.443342 | 1.444024 | 1.444705 |
| 85 | 1.445388 | 1.446071 | 1.446754 | 1.447438 | 1.448121 | 1.448806 | 1.449491 | 1.450175 | 1.450860 | 1.451545 |
| 86 | 1.452232 | 1.452919 | 1.453605 | 1.454292 | 1.454980 | 1.455668 | 1.456357 | 1.457045 | 1.457735 | 1.458424 |
| 87 | 1.459114 | 1.459805 | 1.460495 | 1.461186 | 1.461877 | 1.462568 | 1.463260 | 1.463953 | 1.464645 | 1.465338 |
| 88 | 1.466032 | 1.466726 | 1.467420 | 1.468115 | 1.468810 | 1.469504 | 1.470200 | 1.470896 | 1.471592 | 1.472289 |
| 89 | 1.472986 | 1.473684 | 1.474381 | 1.475080 | 1.475779 | 1.476477 | 1.477176 | 1.477876 | 1.478575 | 1.479275 |
| 90 | 1.479976 | 1.480677 | 1.481378 | 1.482080 | 1.482782 | 1.483484 | 1.484187 | 1.484890 | 1.485593 | 1.486297 |
| 91 | 1.487002 | 1.487707 | 1.488411 | 1.489117 | 1.489823 | 1.490528 | 1.491234 | 1.491941 | 1.492647 | 1.493355 |
| 92 | 1.494063 | 1.494771 | 1.495479 | 1.496188 | 1.496897 | 1.497606 | 1.498316 | 1.499026 | 1.499736 | 1.500447 |
| 93 | 1.501158 | 1.501870 | 1.502582 | 1.503293 | 1.504006 | 1.504719 | 1.505432 | 1.506146 | 1.506859 | 1.507574 |
| 94 | 1.508289 | 1.509004 | 1.509720 | 1.510435 | 1.511151 | 1.511868 | 1.512585 | 1.513302 | 1.514019 | 1.514737 |
| 95 | 1.515455 | 1.516174 | 1.516893 | 1.517612 | 1.518332 | 1.519051 | 1.519771 | 1.520492 | 1.521212 | 1.521934 |
| 96 | 1.522656 | 1.523378 | 1.524100 | 1.524823 | 1.525546 | 1.526269 | 1.526993 | 1.527717 | 1.528441 | 1.529166 |
| 97 | 1.529891 | 1.530616 | 1.531342 | 1.532068 | 1.532794 | 1.533521 | 1.534248 | 1.534976 | 1.535704 | 1.536432 |
| 98 | 1.537161 | 1.537889 | 1.538618 | 1.539347 | 1.540076 | 1.540806 | 1.541536 | 1.542267 | 1.542998 | 1.543730 |
| 99 | 1.544462 | 1.545194 | 1.545926 | 1.546659 | 1.547392 | 1.548127 | 1.548861 | 1.549595 | 1.550329 | 1.551064 |
| 100 | 1.551800 | | | | | | | | | |

## TABLE* 2.

### Temperature Corrections for Changing Percentages of Sugar by Specific Gravity to True Values at 20°C.

| Temperature. Degrees Centigrade. | Observed per cent of sugar. 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Correction to be subtracted from observed per cent. | | | | | | | | | | | | | |
| 0 | 0.30 | 0.49 | 0.65 | 0.77 | 0.89 | 0.99 | 1.08 | 1.16 | 1.24 | 1.31 | 1.37 | 1.41 | 1.44 | 1.49 |
| 5 | 0.36 | 0.47 | 0.56 | 0.65 | 0.73 | 0.80 | 0.86 | 0.91 | 0.97 | 1.01 | 1.05 | 1.08 | 1.10 | 1.14 |
| 10 | 0.32 | 0.38 | 0.43 | 0.48 | 0.52 | 0.57 | 0.60 | 0.64 | 0.67 | 0.70 | 0.72 | 0.74 | 0.75 | 0.77 |
| 11 | 0.31 | 0.35 | 0.40 | 0.44 | 0.48 | 0.51 | 0.55 | 0.58 | 0.60 | 0.63 | 0.65 | 0.66 | 0.68 | 0.70 |
| 12 | 0.29 | 0.32 | 0.36 | 0.40 | 0.43 | 0.46 | 0.50 | 0.52 | 0.54 | 0.56 | 0.58 | 0.59 | 0.60 | 0.62 |
| 13 | 0.26 | 0.29 | 0.32 | 0.35 | 0.38 | 0.41 | 0.44 | 0.46 | 0.48 | 0.49 | 0.51 | 0.52 | 0.53 | 0.55 |
| 14 | 0.24 | 0.26 | 0.29 | 0.31 | 0.34 | 0.36 | 0.38 | 0.40 | 0.41 | 0.42 | 0.44 | 0.45 | 0.46 | 0.47 |
| 15 | 0.20 | 0.22 | 0.24 | 0.26 | 0.28 | 0.30 | 0.32 | 0.33 | 0.34 | 0.36 | 0.36 | 0.37 | 0.38 | 0.39 |
| 16 | 0.17 | 0.18 | 0.20 | 0.22 | 0.23 | 0.25 | 0.26 | 0.27 | 0.28 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 |
| 17 | 0.13 | 0.14 | 0.15 | 0.16 | 0.18 | 0.19 | 0.20 | 0.20 | 0.21 | 0.21 | 0.22 | 0.23 | 0.23 | 0.24 |
| 18 | 0.09 | 0.10 | 0.10 | 0.11 | 0.12 | 0.13 | 0.13 | 0.14 | 0.14 | 0.14 | 0.15 | 0.15 | 0.15 | 0.16 |
| 19 | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 |
| | Correction to be added to observed per cent. | | | | | | | | | | | | | |
| 21 | 0.04 | 0.05 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | 0.09 |
| 22 | 0.10 | 0.10 | 0.11 | 0.12 | 0.12 | 0.13 | 0.14 | 0.14 | 0.15 | 0.15 | 0.16 | 0.16 | 0.16 | 0.16 |
| 23 | 0.16 | 0.16 | 0.17 | 0.17 | 0.19 | 0.20 | 0.21 | 0.21 | 0.22 | 0.23 | 0.24 | 0.24 | 0.24 | 0.24 |
| 24 | 0.21 | 0.22 | 0.23 | 0.24 | 0.26 | 0.27 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 | 0.32 | 0.32 | 0.32 |
| 25 | 0.27 | 0.28 | 0.30 | 0.31 | 0.32 | 0.34 | 0.35 | 0.36 | 0.38 | 0.38 | 0.39 | 0.39 | 0.40 | 0.39 |
| 26 | 0.33 | 0.34 | 0.36 | 0.37 | 0.40 | 0.40 | 0.42 | 0.44 | 0.46 | 0.47 | 0.47 | 0.48 | 0.48 | 0.48 |
| 27 | 0.40 | 0.41 | 0.42 | 0.44 | 0.46 | 0.48 | 0.50 | 0.52 | 0.54 | 0.54 | 0.55 | 0.56 | 0.56 | 0.56 |
| 28 | 0.46 | 0.47 | 0.49 | 0.51 | 0.54 | 0.56 | 0.58 | 0.60 | 0.61 | 0.62 | 0.63 | 0.64 | 0.64 | 0.64 |
| 29 | 0.54 | 0.55 | 0.56 | 0.59 | 0.61 | 0.63 | 0.66 | 0.68 | 0.70 | 0.70 | 0.71 | 0.72 | 0.72 | 0.72 |
| 30 | 0.61 | 0.62 | 0.63 | 0.66 | 0.68 | 0.71 | 0.73 | 0.76 | 0.78 | 0.78 | 0.79 | 0.80 | 0.80 | 0.81 |
| 35 | 0.99 | 1.01 | 1.02 | 1.06 | 1.10 | 1.13 | 1.16 | 1.18 | 1.20 | 1.21 | 1.22 | 1.22 | 1.23 | 1.22 |
| 40 | 1.42 | 1.45 | 1.47 | 1.51 | 1.54 | 1.57 | 1.60 | 1.62 | 1.64 | 1.65 | 1.65 | 1.65 | 1.66 | 1.65 |
| 45 | 1.91 | 1.94 | 1.96 | 2.00 | 2.03 | 2.05 | 2.07 | 2.09 | 2.10 | 2.10 | 2.10 | 2.10 | 2.10 | 2.08 |
| 50 | 2.46 | 2.48 | 2.50 | 2.53 | 2.56 | 2.57 | 2.58 | 2.59 | 2.59 | 2.58 | 2.58 | 2.57 | 2.56 | 2.52 |
| 55 | 3.05 | 3.07 | 3.09 | 3.12 | 3.12 | 3.12 | 3.12 | 3.11 | 3.10 | 3.08 | 3.07 | 3.05 | 3.03 | 2.97 |
| 60 | 3.69 | 3.72 | 3.73 | 3.73 | 3.72 | 3.70 | 3.67 | 3.65 | 3.62 | 3.60 | 3.57 | 3.54 | 3.50 | 3.43 |

* Taken from Circular 19, 1909, U. S. Bureau of Standards. The data of the Kaiserliche Normal-Eichungs-Kommission were used in making the calculations, the specific gravity instrument being assumed to be of Jena 16III glass. On account of the differences in cubical expansion of glass the corrections must be used with caution for temperatures much different from 20° C. *See also* "Handbook," page 31.

## TABLE * 3.

SPECIFIC GRAVITY OF SUCROSE SOLUTIONS AT $\frac{17.5°}{17.5°}$ C. WITH CORRESPONDING DEGREES BRIX AND BAUMÉ

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 0.0 | 1.00000 | 0.0 | 0.0 | 4.8 | 1.01890 | 2.7 | 2.7 |
| 0.1 | 1.00038 | 0.1 | 0.1 | 4.9 | 1.01930 | 2.8 | 2.7 |
| 0.2 | 1.00077 | 0.1 | 0.1 | **5.0** | 1.01970 | 2.8 | 2.8 |
| 0.3 | 1.00116 | 0.2 | 0.2 | 5.1 | 1.02010 | 2.9 | 2.8 |
| 0.4 | 1.00155 | 0.2 | 0.2 | 5.2 | 1.02051 | 2.95 | 2.9 |
| 0.5 | 1.00193 | 0.3 | 0.3 | 5.3 | 1.02091 | 3.0 | 2.9 |
| 0.6 | 1.00232 | 0.3 | 0.3 | 5.4 | 1.02131 | 3.1 | 3.0 |
| 0.7 | 1.00271 | 0.4 | 0.4 | 5.5 | 1.02171 | 3.1 | 3.0 |
| 0.8 | 1.00310 | 0.45 | 0.4 | 5.6 | 1.02211 | 3.2 | 3.1 |
| 0.9 | 1.00349 | 0.5 | 0.5 | 5.7 | 1.02252 | 3.2 | 3.2 |
| **1.0** | 1.00388 | 0.6 | 0.55 | 5.8 | 1.02292 | 3.3 | 3.2 |
| 1.1 | 1.00427 | 0.6 | 0.6 | 5.9 | 1.02333 | 3.35 | 3.3 |
| 1.2 | 1.00466 | 0.7 | 0.7 | **6.0** | 1.02373 | 3.4 | 3.3 |
| 1.3 | 1.00505 | 0.7 | 0.7 | 6.1 | 1.02413 | 3.5 | 3.4 |
| 1.4 | 1.00544 | 0.8 | 0.8 | 6.2 | 1.02454 | 3.5 | 3.4 |
| 1.5 | 1.00583 | 0.85 | 0.8 | 6.3 | 1.02494 | 3.6 | 3.5 |
| 1.6 | 1.00622 | 0.9 | 0.9 | 6.4 | 1.02535 | 3.6 | 3.6 |
| 1.7 | 1.00662 | 1.0 | 0.9 | 6.5 | 1.02575 | 3.7 | 3.6 |
| 1.8 | 1.00701 | 1.0 | 1.0 | 6.6 | 1.02616 | 3.7 | 3.7 |
| 1.9 | 1.00740 | 1.1 | 1.05 | 6.7 | 1.02657 | 3.8 | 3.7 |
| **2.0** | 1.00779 | 1.1 | 1.1 | 6.8 | 1.02697 | 3.9 | 3.8 |
| 2.1 | 1.00818 | 1.2 | 1.2 | 6.9 | 1.02738 | 3.9 | 3.8 |
| 2.2 | 1.00858 | 1.2 | 1.2 | **7.0** | 1.02779 | 4.0 | 3.9 |
| 2.3 | 1.00897 | 1.3 | 1.3 | 7.1 | 1.02819 | 4.0 | 3.9 |
| 2.4 | 1.00936 | 1.4 | 1.3 | 7.2 | 1.02860 | 4.1 | 4.0 |
| 2.5 | 1.00976 | 1.4 | 1.4 | 7.3 | 1.02901 | 4.1 | 4.1 |
| 2.6 | 1.01015 | 1.5 | 1.4 | 7.4 | 1.02942 | 4.2 | 4.1 |
| 2.7 | 1.01055 | 1.5 | 1.5 | 7.5 | 1.02983 | 4.25 | 4.2 |
| 2.8 | 1.01094 | 1.6 | 1.55 | 7.6 | 1.03024 | 4.3 | 4.2 |
| 2.9 | 1.01134 | 1.6 | 1.6 | 7.7 | 1.03064 | 4.4 | 4.3 |
| **3.0** | 1.01173 | 1.7 | 1.7 | 7.8 | 1.03105 | 4.4 | 4.3 |
| 3.1 | 1.01213 | 1.8 | 1.7 | 7.9 | 1.03146 | 4.5 | 4.4 |
| 3.2 | 1.01252 | 1.8 | 1.8 | **8.0** | 1.03187 | 4.5 | 4.4 |
| 3.3 | 1.01292 | 1.9 | 1.8 | 8.1 | 1.03228 | 4.6 | 4.5 |
| 3.4 | 1.01332 | 1.9 | 1.9 | 8.2 | 1.03270 | 4.6 | 4.6 |
| 3.5 | 1.01371 | 2.0 | 1.9 | 8.3 | 1.03311 | 4.7 | 4.6 |
| 3.6 | 1.01411 | 2.0 | 2.0 | 8.4 | 1.03352 | 4.8 | 4.7 |
| 3.7 | 1.01451 | 2.1 | 2.0 | 8.5 | 1.03393 | 4.8 | 4.7 |
| 3.8 | 1.01491 | 2.2 | 2.1 | 8.6 | 1.03434 | 4.9 | 4.8 |
| 3.9 | 1.01531 | 2.2 | 2.2 | 8.7 | 1.03475 | 4.9 | 4.8 |
| **4.0** | 1.01570 | 2.3 | 2.2 | 8.8 | 1.03517 | 5.0 | 4.9 |
| 4.1 | 1.01610 | 2.3 | 2.3 | 8.9 | 1.03558 | 5.0 | 4.9 |
| 4.2 | 1.01650 | 2.4 | 2.3 | **9.0** | 1.03599 | 5.1 | 5.0 |
| 4.3 | 1.01690 | 2.4 | 2.4 | 9.1 | 1.03640 | 5.2 | 5.05 |
| 4.4 | 1.01730 | 2.5 | 2.4 | 9.2 | 1.03682 | 5.2 | 5.1 |
| 4.5 | 1.01770 | 2.55 | 2.5 | 9.3 | 1.03723 | 5.3 | 5.2 |
| 4.6 | 1.01810 | 2.6 | 2.6 | 9.4 | 1.03765 | 5.3 | 5.2 |
| 4.7 | 1.01850 | 2.7 | 2.6 | 9.5 | 1.03806 | 5.4 | 5.3 |

* *See* "Handbook," pages 29 and 48.

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 9.6 | 1.03848 | 5.4 | 5.3 | 14.8 | 1.06047 | 8.4 | 8.2 |
| 9.7 | 1.03889 | 5.5 | 5.4 | 14.9 | 1.06090 | 8.4 | 8.3 |
| 9.8 | 1.03931 | 5.55 | 5.4 | **15.0** | 1.06133 | 8.5 | 8.3 |
| 9.9 | 1.03972 | 5.6 | 5.5 | 15.1 | 1.06176 | 8.5 | 8.4 |
| **10.0** | 1.04014 | 5.7 | 5.55 | 15.2 | 1.06219 | 8.55 | 8.4 |
| 10.1 | 1.04055 | 5.7 | 5.6 | 15.3 | 1.06262 | 8.6 | 8.5 |
| 10.2 | 1.04097 | 5.8 | 5.7 | 15.4 | 1.06306 | 8.7 | 8.5 |
| 10.3 | 1.04139 | 5.8 | 5.7 | 15.5 | 1.06349 | 8.8 | 8.6 |
| 10.4 | 1.04180 | 5.9 | 5.8 | 15.6 | 1.06392 | 8.8 | 8.65 |
| 10.5 | 1.04222 | 5.9 | 5.8 | 15.7 | 1.06436 | 8.9 | 8.7 |
| 10.6 | 1.04264 | 6.0 | 5.9 | 15.8 | 1.06479 | 8.9 | 8.8 |
| 10.7 | 1.04306 | 6.1 | 5.9 | 15.9 | 1.06522 | 9.0 | 8.8 |
| 10.8 | 1.04348 | 6.1 | 6.0 | **16.0** | 1.06566 | 9.0 | 8.9 |
| 10.9 | 1.04390 | 6.2 | 6.05 | 16.1 | 1.06609 | 9.1 | 8.9 |
| **11.0** | 1.04431 | 6.2 | 6.1 | 16.2 | 1.06653 | 9.2 | 9.0 |
| 11.1 | 1.04473 | 6.3 | 6.2 | 16.3 | 1.06696 | 9.2 | 9.0 |
| 11.2 | 1.04515 | 6.3 | 6.2 | 16.4 | 1.06740 | 9.3 | 9.1 |
| 11.3 | 1.04557 | 6.4 | 6.3 | 16.5 | 1.06783 | 9.3 | 9.1 |
| 11.4 | 1.04599 | 6.5 | 6.3 | 16.6 | 1.06827 | 9.4 | 9.2 |
| 11.5 | 1.04641 | 6.5 | 6.4 | 16.7 | 1.06871 | 9.4 | 9.25 |
| 11.6 | 1.04683 | 6.6 | 6.4 | 16.8 | 1.06914 | 9.5 | 9.3 |
| 11.7 | 1.04726 | 6.6 | 6.5 | 16.9 | 1.06958 | 9.5 | 9.4 |
| 11.8 | 1.04768 | 6.7 | 6.55 | **17.0** | 1.07002 | 9.6 | 9.4 |
| 11.9 | 1.04810 | 6.7 | 6.6 | 17.1 | 1.07046 | 9.7 | 9.5 |
| **12.0** | 1.04852 | 6.8 | 6.7 | 17.2 | 1.07090 | 9.7 | 9.5 |
| 12.1 | 1.04894 | 6.8 | 6.7 | 17.3 | 1.07133 | 9.8 | 9.6 |
| 12.2 | 1.04937 | 6.9 | 6.8 | 17.4 | 1.07177 | 9.8 | 9.6 |
| 12.3 | 1.04979 | 7.0 | 6.8 | 17.5 | 1.07221 | 9.9 | 9.7 |
| 12.4 | 1.05021 | 7.0 | 6.9 | 17.6 | 1.07265 | 9.9 | 9.75 |
| 12.5 | 1.05064 | 7.1 | 6.9 | 17.7 | 1.07309 | 10.0 | 9.8 |
| 12.6 | 1.05106 | 7.1 | 7.0 | 17.8 | 1.07353 | 10.0 | 9.9 |
| 12.7 | 1.05149 | 7.2 | 7.05 | 17.9 | 1.07397 | 10.1 | 9.9 |
| 12.8 | 1.05191 | 7.2 | 7.1 | **18.0** | 1.07441 | 10.1 | 10.0 |
| 12.9 | 1.05233 | 7.3 | 7.2 | 18.1 | 1.07485 | 10.2 | 10.0 |
| **13.0** | 1.05276 | 7.4 | 7.2 | 18.2 | 1.07530 | 10.3 | 10.1 |
| 13.1 | 1.05318 | 7.4 | 7.3 | 18.3 | 1.07574 | 10.3 | 10.1 |
| 13.2 | 1.05361 | 7.5 | 7.3 | 18.4 | 1.07618 | 10.4 | 10.2 |
| 13.3 | 1.05404 | 7.5 | 7.4 | 18.5 | 1.07662 | 10.4 | 10.2 |
| 13.4 | 1.05446 | 7.6 | 7.4 | 18.6 | 1.07706 | 10.5 | 10.3 |
| 13.5 | 1.05489 | 7.6 | 7.5 | 18.7 | 1.07751 | 10.5 | 10.35 |
| 13.6 | 1.05532 | 7.7 | 7.5 | 18.8 | 1.07795 | 10.6 | 10.4 |
| 13.7 | 1.05574 | 7.75 | 7.6 | 18.9 | 1.07839 | 10.6 | 10.5 |
| 13.8 | 1.05617 | 7.8 | 7.65 | **19.0** | 1.07884 | 10.7 | 10.5 |
| 13.9 | 1.05660 | 7.9 | 7.7 | 19.1 | 1.07928 | 10.8 | 10.6 |
| **14.0** | 1.05703 | 7.9 | 7.8 | 19.2 | 1.07973 | 10.8 | 10.6 |
| 14.1 | 1.05746 | 8.0 | 7.8 | 19.3 | 1.08017 | 10.9 | 10.7 |
| 14.2 | 1.05789 | 8.0 | 7.9 | 19.4 | 1.08062 | 10.9 | 10.7 |
| 14.3 | 1.05831 | 8.1 | 7.9 | 19.5 | 1.08106 | 11.0 | 10.8 |
| 14.4 | 1.05874 | 8.1 | 8.0 | 19.6 | 1.08151 | 11.1 | 10.85 |
| 14.5 | 1.05917 | 8.2 | 8.0 | 19.7 | 1.08196 | 11.1 | 10.9 |
| 14.6 | 1.05960 | 8.3 | 8.1 | 19.8 | 1.08240 | 11.2 | 11.0 |
| 14.7 | 1.06003 | 8.3 | 8.15 | 19.9 | 1.08285 | 11.2 | 11.0 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| **20.0** | 1.08329 | 11.3 | 11.1 | 25.2 | 1.10700 | 14.2 | 13.9 |
| 20.1 | 1.08374 | 11.3 | 11.1 | 25.3 | 1.10746 | 14.2 | 14.0 |
| 20.2 | 1.08419 | 11.4 | 11.2 | 25.4 | 1.10793 | 14.3 | 14.0 |
| 20.3 | 1.08464 | 11.5 | 11.2 | 25.5 | 1.10839 | 14.3 | 14.1 |
| 20.4 | 1.08509 | 11.5 | 11.3 | 25.6 | 1.10886 | 14.4 | 14.1 |
| 20.5 | 1.08553 | 11.6 | 11.3 | 25.7 | 1.10932 | 14.5 | 14.2 |
| 20.6 | 1.08599 | 11.6 | 11.4 | 25.8 | 1.10979 | 14.5 | 14.2 |
| 20.7 | 1.08643 | 11.7 | 11.45 | 25.9 | 1.11026 | 14.6 | 14.3 |
| 20.8 | 1.08688 | 11.7 | 11.5 | **26.0** | 1.11072 | 14.6 | 14.35 |
| 20.9 | 1.08733 | 11.8 | 11.6 | 26.1 | 1.11119 | 14.7 | 14.4 |
| **21.0** | 1.08778 | 11.8 | 11.6 | 26.2 | 1.11166 | 14.7 | 14.5 |
| 21.1 | 1.08824 | 11.9 | 11.7 | 26.3 | 1.11213 | 14.8 | 14.5 |
| 21.2 | 1.08869 | 11.95 | 11.7 | 26.4 | 1.11259 | 14.85 | 14.6 |
| 21.3 | 1.08914 | 12.0 | 11.8 | 26.5 | 1.11306 | 14.9 | 14.6 |
| 21.4 | 1.08959 | 12.0 | 11.8 | 26.6 | 1.11353 | 15.0 | 14.7 |
| 21.5 | 1.09004 | 12.1 | 11.9 | 26.7 | 1.11400 | 15.0 | 14.7 |
| 21.6 | 1.09049 | 12.1 | 11.95 | 26.8 | 1.11447 | 15.1 | 14.8 |
| 21.7 | 1.09095 | 12.2 | 12.0 | 26.9 | 1.11494 | 15.1 | 14.8 |
| 21.8 | 1.09140 | 12.3 | 12.05 | **27.0** | 1.11541 | 15.2 | 14.9 |
| 21.9 | 1.09185 | 12.3 | 12.1 | 27.1 | 1.11588 | 15.2 | 14.9 |
| **22.0** | 1.09231 | 12.4 | 12.2 | 27.2 | 1.11635 | 15.3 | 15.0 |
| 22.1 | 1.09276 | 12.5 | 12.2 | 27.3 | 1.11682 | 15.3 | 15.1 |
| 22.2 | 1.09321 | 12.5 | 12.3 | 27.4 | 1.11729 | 15.4 | 15.1 |
| 22.3 | 1.09367 | 12.6 | 12.3 | 27.5 | 1.11776 | 15.5 | 15.2 |
| 22.4 | 1.09412 | 12.6 | 12.4 | 27.6 | 1.11824 | 15.5 | 15.2 |
| 22.5 | 1.09458 | 12.7 | 12.4 | 27.7 | 1.11871 | 15.6 | 15.3 |
| 22.6 | 1.09503 | 12.7 | 12.5 | 27.8 | 1.11918 | 15.6 | 15.3 |
| 22.7 | 1.09549 | 12.8 | 12.55 | 27.9 | 1.11965 | 15.7 | 15.4 |
| 22.8 | 1.09595 | 12.85 | 12.6 | **28.0** | 1.12013 | 15.7 | 15.4 |
| 22.9 | 1.09640 | 12.9 | 12.7 | 28.1 | 1.12060 | 15.8 | 15.5 |
| **23.0** | 1.09686 | 13.0 | 12.7 | 28.2 | 1.12107 | 15.8 | 15.55 |
| 23.1 | 1.09732 | 13.0 | 12.8 | 28.3 | 1.12155 | 15.9 | 15.6 |
| 23.2 | 1.09777 | 13.1 | 12.8 | 28.4 | 1.12202 | 16.0 | 15.7 |
| 23.3 | 1.09823 | 13.1 | 12.9 | 28.5 | 1.12250 | 16.0 | 15.7 |
| 23.4 | 1.09869 | 13.2 | 12.9 | 28.6 | 1.12297 | 16.1 | 15.8 |
| 23.5 | 1.09915 | 13.2 | 13.0 | 28.7 | 1.12345 | 16.1 | 15.8 |
| 23.6 | 1.09961 | 13.3 | 13.0 | 28.8 | 1.12393 | 16.2 | 15.9 |
| 23.7 | 1.10007 | 13.3 | 13.1 | 28.9 | 1.12440 | 16.2 | 15.9 |
| 23.8 | 1.10053 | 13.4 | 13.15 | **29.0** | 1.12488 | 16.3 | 16.0 |
| 23.9 | 1.10099 | 13.5 | 13.2 | 29.1 | 1.12536 | 16.3 | 16.0 |
| **24.0** | 1.10145 | 13.5 | 13.3 | 29.2 | 1.12583 | 16.4 | 16.1 |
| 24.1 | 1.10191 | 13.6 | 13.3 | 29.3 | 1.12631 | 16.5 | 16.1 |
| 24.2 | 1.10237 | 13.6 | 13.4 | 29.4 | 1.12679 | 16.5 | 16.2 |
| 24.3 | 1.10283 | 13.7 | 13.4 | 29.5 | 1.12727 | 16.6 | 16.25 |
| 24.4 | 1.10329 | 13.7 | 13.5 | 29.6 | 1.12775 | 16.6 | 16.3 |
| 24.5 | 1.10375 | 13.8 | 13.5 | 29.7 | 1.12823 | 16.7 | 16.4 |
| 24.6 | 1.10421 | 13.8 | 13.6 | 29.8 | 1.12871 | 16.7 | 16.4 |
| 24.7 | 1.10468 | 13.9 | 13.6 | 29.9 | 1.12919 | 16.8 | 16.5 |
| 24.8 | 1.10514 | 14.0 | 13.7 | **30.0** | 1.12967 | 16.8 | 16.5 |
| 24.9 | 1.10560 | 14.0 | 13.75 | 30.1 | 1.13015 | 16.9 | 16.6 |
| **25.0** | 1.10607 | 14.1 | 13.8 | 30.2 | 1.13063 | 16.95 | 16.6 |
| 25.1 | 1.10653 | 14.1 | 13.9 | 30.3 | 1.13111 | 17.0 | 16.7 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old |
| 30.4 | 1.13159 | 17.1 | 16.7 | 35.6 | 1.15710 | 19.9 | 19.55 |
| 30.5 | 1.13207 | 17.1 | 16.8 | 35.7 | 1.15760 | 20.0 | 19.6 |
| 30.6 | 1.13255 | 17.2 | 16.85 | 35.8 | 1.15810 | 20.0 | 19.65 |
| 30.7 | 1.13304 | 17.2 | 16.9 | 35.9 | 1.15861 | 20.1 | 19.7 |
| 30.8 | 1.13352 | 17.3 | 17.0 | **36.0** | 1.15911 | 20.1 | 19.8 |
| 30.9 | 1.13400 | 17.3 | 17.0 | 36.1 | 1.15961 | 20.2 | 19.8 |
| **31.0** | 1.13449 | 17.4 | 17.1 | 36.2 | 1.16011 | 20.25 | 19.9 |
| 31.1 | 1.13497 | 17.45 | 17.1 | 36.3 | 1.16061 | 20.3 | 19.9 |
| 31.2 | 1.13545 | 17.5 | 17.2 | 36.4 | 1.16111 | 20.4 | 20.0 |
| 31.3 | 1.13594 | 17.6 | 17.2 | 36.5 | 1.16162 | 20.4 | 20.0 |
| 31.4 | 1.13642 | 17.6 | 17.3 | 36.6 | 1.16212 | 20.5 | 20.1 |
| 31.5 | 1.13691 | 17.7 | 17.3 | 36.7 | 1.16262 | 20.5 | 20.1 |
| 31.6 | 1.13740 | 17.7 | 17.4 | 36.8 | 1.16313 | 20.6 | 20.2 |
| 31.7 | 1.13788 | 17.8 | 17.4 | 36.9 | 1.16363 | 20.6 | 20.2 |
| 31.8 | 1.13837 | 17.8 | 17.5 | **37.0** | 1.16413 | 20.7 | 20.3 |
| 31.9 | 1.13885 | 17.9 | 17.55 | 37.1 | 1.16464 | 20.7 | 20.35 |
| **32.0** | 1.13934 | 17.95 | 17.6 | 37.2 | 1.16514 | 20.8 | 20.4 |
| 32.1 | 1.13983 | 18.0 | 17.7 | 37.3 | 1.16565 | 20.9 | 20.5 |
| 32.2 | 1.14032 | 18.0 | 17.7 | 37.4 | 1.16616 | 20.9 | 20.5 |
| 32.3 | 1.14081 | 18.1 | 17.8 | 37.5 | 1.16666 | 21.0 | 20.6 |
| 32.4 | 1.14129 | 18.2 | 17.8 | 37.6 | 1.16717 | 21.0 | 20.6 |
| 32.5 | 1.14178 | 18.2 | 17.9 | 37.7 | 1.16768 | 21.1 | 20.7 |
| 32.6 | 1.14227 | 18.3 | 17.9 | 37.8 | 1.16818 | 21.1 | 20.7 |
| 32.7 | 1.14276 | 18.3 | 18.0 | 37.9 | 1.16869 | 21.2 | 20.8 |
| 32.8 | 1.14325 | 18.4 | 18.0 | **38.0** | 1.16920 | 21.2 | 20.8 |
| 32.9 | 1.14374 | 18.4 | 18.1 | 38.1 | 1.16971 | 21.3 | 20.9 |
| **33.0** | 1.14423 | 18.5 | 18.15 | 38.2 | 1.17022 | 21.35 | 20.9 |
| 33.1 | 1.14472 | 18.55 | 18.2 | 38.3 | 1.17072 | 21.4 | 21.0 |
| 33.2 | 1.14521 | 18.6 | 18.25 | 38.4 | 1.17123 | 21.5 | 21.05 |
| 33.3 | 1.14570 | 18.7 | 18.3 | 38.5 | 1.17174 | 21.5 | 21.1 |
| 33.4 | 1.14620 | 18.7 | 18.4 | 38.6 | 1.17225 | 21.6 | 21.15 |
| 33.5 | 1.14669 | 18.8 | 18.4 | 38.7 | 1.17276 | 21.6 | 21.2 |
| 33.6 | 1.14718 | 18.8 | 18.5 | 38.8 | 1.17327 | 21.7 | 21.3 |
| 33.7 | 1.14767 | 18.9 | 18.5 | 38.9 | 1.17379 | 21.7 | 21.3 |
| 33.8 | 1.14817 | 18.9 | 18.6 | **39.0** | 1.17430 | 21.8 | 21.4 |
| 33.9 | 1.14866 | 19.0 | 18.6 | 39.1 | 1.17481 | 21.8 | 21.4 |
| **34.0** | 1.14915 | 19.05 | 18.7 | 39.2 | 1.17532 | 21.9 | 21.5 |
| 34.1 | 1.14965 | 19.1 | 18.7 | 39.3 | 1.17583 | 21.9 | 21.5 |
| 34.2 | 1.15014 | 19.2 | 18.8 | 39.4 | 1.17635 | 22.0 | 21.6 |
| 34.3 | 1.15064 | 19.2 | 18.85 | 39.5 | 1.17686 | 22.05 | 21.6 |
| 34.4 | 1.15113 | 19.3 | 18.9 | 39.6 | 1.17737 | 22.1 | 21.7 |
| 34.5 | 1.15163 | 19.3 | 18.95 | 39.7 | 1.17789 | 22.2 | 21.7 |
| 34.6 | 1.15213 | 19.4 | 19.0 | 39.8 | 1.17840 | 22.2 | 21.8 |
| 34.7 | 1.15262 | 19.4 | 19.1 | 39.9 | 1.17892 | 22.3 | 21.85 |
| 34.8 | 1.15312 | 19.5 | 19.1 | **40.0** | 1.17943 | 22.3 | 21.9 |
| 34.9 | 1.15362 | 19.5 | 19.2 | 40.1 | 1.17995 | 22.4 | 22.0 |
| **35.0** | 1.15411 | 19.6 | 19.2 | 40.2 | 1.18046 | 22.4 | 22.0 |
| 35.1 | 1.15461 | 19.65 | 19.3 | 40.3 | 1.18098 | 22.5 | 22.1 |
| 35.2 | 1.15511 | 19.7 | 19.3 | 40.4 | 1.18150 | 22.5 | 22.1 |
| 35.3 | 1.15561 | 19.8 | 19.4 | 40.5 | 1.18201 | 22.6 | 22.2 |
| 35.4 | 1.15611 | 19.8 | 19.4 | 40.6 | 1.18253 | 22.6 | 22.2 |
| 35.5 | 1.15661 | 19.9 | 19.5 | 40.7 | 1.18305 | 22.7 | 22.3 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 40.8 | 1.18357 | 22.8 | 22.3 | **46.0** | 1.21100 | 25.6 | 25.1 |
| 40.9 | 1.18408 | 22.8 | 22.4 | 46.1 | 1.21154 | 25.6 | 25.1 |
| **41.0** | 1.18460 | 22.9 | 22.4 | 46.2 | 1.21208 | 25.7 | 25.2 |
| 41.1 | 1.18512 | 22.9 | 22.5 | 46.3 | 1.21261 | 25.7 | 25.2 |
| 41.2 | 1.18564 | 23.0 | 22.5 | 46.4 | 1.21315 | 25.8 | 25.3 |
| 41.3 | 1.18616 | 23.0 | 22.6 | 46.5 | 1.21369 | 25.8 | 25.35 |
| 41.4 | 1.18668 | 23.1 | 22.65 | 46.6 | 1.21423 | 25.9 | 25.4 |
| 41.5 | 1.18720 | 23.1 | 22.7 | 46.7 | 1.21477 | 25.95 | 25.45 |
| 41.6 | 1.18772 | 23.2 | 22.75 | 46.8 | 1.21531 | 26.0 | 25.5 |
| 41.7 | 1.18824 | 23.25 | 22.8 | 46.9 | 1.21585 | 26.1 | 25.6 |
| 41.8 | 1.18877 | 23.3 | 22.9 | **47.0** | 1.21639 | 26.1 | 25.6 |
| 41.9 | 1.18929 | 23.4 | 22.9 | 47.1 | 1.21693 | 26.2 | 25.7 |
| **42.0** | 1.18981 | 23.4 | 23.0 | 47.2 | 1.21747 | 26.2 | 25.7 |
| 42.1 | 1.19033 | 23.5 | 23.0 | 47.3 | 1.21802 | 26.3 | 25.8 |
| 42.2 | 1.19086 | 23.5 | 23.1 | 47.4 | 1.21856 | 26.3 | 25.8 |
| 42.3 | 1.19138 | 23.6 | 23.1 | 47.5 | 1.21910 | 26.4 | 25.9 |
| 42.4 | 1.19190 | 23.6 | 23.2 | 47.6 | 1.21964 | 26.4 | 25.9 |
| 42.5 | 1.19243 | 23.7 | 23.2 | 47.7 | 1.22019 | 26.5 | 26.0 |
| 42.6 | 1.19295 | 23.7 | 23.3 | 47.8 | 1.22073 | 26.5 | 26.0 |
| 42.7 | 1.19348 | 23.8 | 23.3 | 47.9 | 1.22127 | 26.6 | 26.1 |
| 42.8 | 1.19400 | 23.8 | 23.4 | **48.0** | 1.22182 | 26.6 | 26.1 |
| 42.9 | 1.19453 | 23.9 | 23.45 | 48.1 | 1.22236 | 26.7 | 26.2 |
| **43.0** | 1.19505 | 23.95 | 23.5 | 48.2 | 1.22291 | 26.75 | 26.2 |
| 43.1 | 1.19558 | 24.0 | 23.55 | 48.3 | 1.22345 | 26.8 | 26.3 |
| 43.2 | 1.19611 | 24.1 | 23.6 | 48.4 | 1.22400 | 26.9 | 26.35 |
| 43.3 | 1.19663 | 24.1 | 23.7 | 48.5 | 1.22455 | 26.9 | 26.4 |
| 43.4 | 1.19716 | 24.2 | 23.7 | 48.6 | 1.22509 | 27.0 | 26.45 |
| 43.5 | 1.19769 | 24.2 | 23.8 | 48.7 | 1.22564 | 27.0 | 26.5 |
| 43.6 | 1.19822 | 24.3 | 23.8 | 48.8 | 1.22619 | 27.1 | 26.6 |
| 43.7 | 1.19875 | 24.3 | 23.9 | 48.9 | 1.22673 | 27.1 | 26.6 |
| 43.8 | 1.19927 | 24.4 | 23.9 | **49.0** | 1.22728 | 27.2 | 26.7 |
| 43.9 | 1.19980 | 24.4 | 24.0 | 49.1 | 1.22783 | 27.2 | 26.7 |
| **44.0** | 1.20033 | 24.5 | 24.0 | 49.2 | 1.22838 | 27.3 | 26.8 |
| 44.1 | 1.20086 | 24.55 | 24.1 | 49.3 | 1.22893 | 27.3 | 26.8 |
| 44.2 | 1.20139 | 24.6 | 24.1 | 49.4 | 1.22948 | 27.4 | 26.9 |
| 44.3 | 1.20192 | 24.65 | 24.2 | 49.5 | 1.23003 | 27.4 | 26.9 |
| 44.4 | 1.20245 | 24.7 | 24.2 | 49.6 | 1.23058 | 27.5 | 27.0 |
| 44.5 | 1.20299 | 24.8 | 24.3 | 49.7 | 1.23113 | 27.6 | 27.0 |
| 44.6 | 1.20352 | 24.8 | 24.35 | 49.8 | 1.23168 | 27.6 | 27.1 |
| 44.7 | 1.20405 | 24.9 | 24.4 | 49.9 | 1.23223 | 27.7 | 27.1 |
| 44.8 | 1.20458 | 24.9 | 24.45 | **50.0** | 1.23278 | 27.7 | 27.2 |
| 44.9 | 1.20512 | 25.0 | 24.5 | 50.1 | 1.23334 | 27.8 | 27.2 |
| **45.0** | 1.20565 | 25.0 | 24.6 | 50.2 | 1.23389 | 27.8 | 27.3 |
| 45.1 | 1.20618 | 25.1 | 24.6 | 50.3 | 1.23444 | 27.9 | 27.3 |
| 45.2 | 1.20672 | 25.1 | 24.7 | 50.4 | 1.23499 | 27.9 | 27.4 |
| 45.3 | 1.20725 | 25.2 | 24.7 | 50.5 | 1.23555 | 28.0 | 27.45 |
| 45.4 | 1.20779 | 25.2 | 24.8 | 50.6 | 1.23610 | 28.0 | 27.5 |
| 45.5 | 1.20832 | 25.3 | 24.8 | 50.7 | 1.23666 | 28.1 | 27.55 |
| 45.6 | 1.20886 | 25.4 | 24.9 | 50.8 | 1.23721 | 28.1 | 27.6 |
| 45.7 | 1.20939 | 25.4 | 24.9 | 50.9 | 1.23777 | 28.2 | 27.7 |
| 45.8 | 1.20993 | 25.5 | 25.0 | **51.0** | 1.23832 | 28.2 | 27.7 |
| 45.9 | 1.21046 | 25.5 | 25.0 | 51.1 | 1.23888 | 28.3 | 27.8 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 51.2 | 1.23943 | 28.35 | 27.8 | 56.4 | 1.26889 | 31.1 | 30.5 |
| 51.3 | 1.23999 | 28.4 | 27.9 | 56.5 | 1.26946 | 31.2 | 30.6 |
| 51.4 | 1.24055 | 28.5 | 27.9 | 56.6 | 1.27004 | 31.2 | 30.6 |
| 51.5 | 1.24111 | 28.5 | 28.0 | 56.7 | 1.27062 | 31.3 | 30.7 |
| 51.6 | 1.24166 | 28.6 | 28.0 | 56.8 | 1.27120 | 31.3 | 30.7 |
| 51.7 | 1.24222 | 28.6 | 28.1 | 56.9 | 1.27177 | 31.4 | 30.8 |
| 51.8 | 1.24278 | 28.7 | 28.1 | **57.0** | 1.27235 | 31.4 | 30.8 |
| 51.9 | 1.24334 | 28.7 | 28.2 | 57.1 | 1.27293 | 31.5 | 30.9 |
| **52.0** | 1.24390 | 28.8 | 28.2 | 57.2 | 1.27351 | 31.5 | 30.9 |
| 52.1 | 1.24446 | 28.8 | 28.3 | 57.3 | 1.27409 | 31.6 | 31.0 |
| 52.2 | 1.24502 | 28.9 | 28.3 | 57.4 | 1.27464 | 31.6 | 31.0 |
| 52.3 | 1.24558 | 28.9 | 28.4 | 57.5 | 1.27525 | 31.7 | 31.1 |
| 52.4 | 1.24614 | 29.0 | 28.4 | 57.6 | 1.27583 | 31.7 | 31.1 |
| 52.5 | 1.24670 | 29.0 | 28.5 | 57.7 | 1.27641 | 31.8 | 31.2 |
| 52.6 | 1.24726 | 29.1 | 28.5 | 57.8 | 1.27699 | 31.8 | 31.2 |
| 52.7 | 1.24782 | 29.15 | 28.6 | 57.9 | 1.27758 | 31.9 | 31.3 |
| 52.8 | 1.24839 | 29.2 | 28.65 | **58.0** | 1.27816 | 31.9 | 31.3 |
| 52.9 | 1.24895 | 29.2 | 28.7 | 58.1 | 1.27874 | 32.0 | 31.4 |
| **53.0** | 1.24951 | 29.3 | 28.75 | 58.2 | 1.27932 | 32.0 | 31.4 |
| 53.1 | 1.25008 | 29.4 | 28.8 | 58.3 | 1.27991 | 32.1 | 31.5 |
| 53.2 | 1.25064 | 29.4 | 28.85 | 58.4 | 1.28049 | 32.15 | 31.5 |
| 53.3 | 1.25120 | 29.5 | 28.9 | 58.5 | 1.28107 | 32.2 | 31.6 |
| 53.4 | 1.25177 | 29.5 | 28.9 | 58.6 | 1.28166 | 32.3 | 31.6 |
| 53.5 | 1.25233 | 29.6 | 29.0 | 58.7 | 1.28224 | 32.3 | 31.7 |
| 53.6 | 1.25290 | 29.6 | 29.1 | 58.8 | 1.28283 | 32.4 | 31.7 |
| 53.7 | 1.25347 | 29.7 | 29.1 | 58.9 | 1.28342 | 32.4 | 31.8 |
| 53.8 | 1.25403 | 29.7 | 29.2 | **59.0** | 1.28400 | 32.5 | 31.85 |
| 53.9 | 1.25460 | 29.8 | 29.2 | 59.1 | 1.28459 | 32.5 | 31.9 |
| **54.0** | 1.25517 | 29.8 | 29.3 | 59.2 | 1.28518 | 32.6 | 31.95 |
| 54.1 | 1.25573 | 29.9 | 29.3 | 59.3 | 1.28576 | 32.6 | 32.0 |
| 54.2 | 1.25630 | 29.9 | 29 4 | 59.4 | 1.28635 | 32.7 | 32.05 |
| 54.3 | 1.25687 | 30.0 | 29.4 | 59.5 | 1.28694 | 32.7 | 32.1 |
| 54.4 | 1.25744 | 30.05 | 29.5 | 59.6 | 1.28753 | 32.8 | 32.15 |
| 54.5 | 1.25801 | 30.1 | 29.5 | 59.7 | 1.28812 | 32.8 | 32.2 |
| 54.6 | 1.25857 | 30.2 | 29.6 | 59.8 | 1.28871 | 32.9 | 32.3 |
| 54.7 | 1.25914 | 30.2 | 29.6 | 59.9 | 1.28930 | 32.9 | 32.3 |
| 54.8 | 1.25971 | 30.3 | 29.7 | **60.0** | 1.28989 | 33.0 | 32.4 |
| 54.9 | 1.26028 | 30.3 | 29.7 | 60.1 | 1.29048 | 33.0 | 32.4 |
| **55.0** | 1.26086 | 30.4 | 29.8 | 60.2 | 1.29107 | 33.1 | 32.5 |
| 55.1 | 1.26143 | 30.4 | 29.8 | 60.3 | 1.29166 | 33.1 | 32.5 |
| 55.2 | 1.26200 | 30.5 | 29.9 | 60.4 | 1.29225 | 33.2 | 32.6 |
| 55.3 | 1.26257 | 30.5 | 29.9 | 60.5 | 1.29284 | 33.2 | 32.6 |
| 55.4 | 1.26314 | 30.6 | 30.0 | 60.6 | 1.29343 | 33.3 | 32.7 |
| 55.5 | 1.26372 | 30.6 | 30.05 | 60.7 | 1.29403 | 33.35 | 32.7 |
| 55.6 | 1.26429 | 30.7 | 30.1 | 60.8 | 1.29462 | 33.4 | 32.8 |
| 55.7 | 1.26486 | 30.7 | 30.15 | 60.9 | 1.29521 | 33.45 | 32.8 |
| 55.8 | 1.26544 | 30.8 | 30.2 | **61.0** | 1.29581 | 33.5 | 32.9 |
| 55.9 | 1.26601 | 30.8 | 30.25 | 61.1 | 1.29640 | 33.6 | 32.9 |
| **56.0** | 1.26658 | 30.9 | 30.3 | 61.2 | 1.29700 | 33.6 | 33.0 |
| 56.1 | 1.26716 | 30.9 | 30.4 | 61.3 | 1.29759 | 33.7 | 33.0 |
| 56.2 | 1.26773 | 31.0 | 30.4 | 61.4 | 1.29819 | 33.7 | 33.1 |
| 56.3 | 1.26831 | 31.05 | 30.5 | 61.5 | 1.29878 | 33.8 | 33.1 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 61.6 | 1.29938 | 33.8 | 33.2 | 66.8 | 1.33093 | 36.5 | 35.8 |
| 61.7 | 1.29998 | 33.9 | 33.2 | 66.9 | 1.33155 | 36.5 | 35.9 |
| 61.8 | 1.30057 | 33.9 | 33.3 | **67.0** | 1.33217 | 36.6 | 35.9 |
| 61.9 | 1.30117 | 34.0 | 33.3 | 67.1 | 1.33278 | 36.6 | 36.0 |
| **62.0** | 1.30177 | 34.0 | 33.4 | 67.2 | 1.33340 | 36.7 | 36.0 |
| 62.1 | 1.30237 | 34.1 | 33.4 | 67.3 | 1.33402 | 36.75 | 36.1 |
| 62.2 | 1.30297 | 34.1 | 33.5 | 67.4 | 1.33464 | 36.8 | 36.1 |
| 62.3 | 1.30356 | 34.2 | 33.5 | 67.5 | 1.33526 | 36.85 | 36.2 |
| 62.4 | 1.30416 | 34.2 | 33.6 | 67.6 | 1.33588 | 36.9 | 36.2 |
| 62.5 | 1.30476 | 34.3 | 33.6 | 67.7 | 1.33650 | 36.95 | 36.3 |
| 62.6 | 1.30536 | 34.3 | 33.7 | 67.8 | 1.33712 | 37.0 | 36.3 |
| 62.7 | 1.30596 | 34.4 | 33.7 | 67.9 | 1.33774 | 37.0 | 36.4 |
| 62.8 | 1.30657 | 34.4 | 33.8 | **68.0** | 1.33836 | 37.1 | 36.4 |
| 62.9 | 1.30717 | 34.5 | 33.8 | 68.1 | 1.33899 | 37.1 | 36.5 |
| **63.0** | 1.30777 | 34.5 | 33.9 | 68.2 | 1.33961 | 37.2 | 36.5 |
| 63.1 | 1.30837 | 34.6 | 33.9 | 68.3 | 1.34023 | 37.3 | 36.6 |
| 63.2 | 1.30897 | 34.6 | 34.0 | 68.4 | 1.34085 | 37.3 | 36.6 |
| 63.3 | 1.30958 | 34.7 | 34.0 | 68.5 | 1.34148 | 37.4 | 36.7 |
| 63.4 | 1.31018 | 34.7 | 34.1 | 68.6 | 1.34210 | 37.4 | 36.7 |
| 63.5 | 1.31078 | 34.8 | 34.1 | 68.7 | 1.34273 | 37.5 | 36.8 |
| 63.6 | 1.31139 | 34.85 | 34.2 | 68.8 | 1.34335 | 37.5 | 36.8 |
| 63.7 | 1.31199 | 34.9 | 34.2 | 68.9 | 1.34398 | 37.6 | 36.9 |
| 63.8 | 1.31260 | 34.95 | 34.3 | **69.0** | 1.34460 | 37.6 | 36.9 |
| 63.9 | 1.31320 | 35.0 | 34.3 | 69.1 | 1.34523 | 37.7 | 37.0 |
| **64.0** | 1.31381 | 35.1 | 34.4 | 69.2 | 1.34585 | 37.7 | 37.0 |
| 64.1 | 1.31442 | 35.1 | 34.4 | 69.3 | 1.34648 | 37.8 | 37.1 |
| 64.2 | 1.31502 | 35.2 | 34.5 | 69.4 | 1.34711 | 37.8 | 37.1 |
| 64.3 | 1.31563 | 35.2 | 34.5 | 69.5 | 1.34774 | 37.9 | 37.2 |
| 64.4 | 1.31624 | 35.3 | 34.6 | 69.6 | 1.34836 | 37.9 | 37.2 |
| 64.5 | 1.31684 | 35.3 | 34.6 | 69.7 | 1.34899 | 38.0 | 37.3 |
| 64.6 | 1.31745 | 35.4 | 34.7 | 69.8 | 1.34962 | 38.0 | 37.3 |
| 64.7 | 1.31806 | 35.4 | 34.7 | 69.9 | 1.35025 | 38.1 | 37.4 |
| 64.8 | 1.31867 | 35.5 | 34.8 | **70.0** | 1.35088 | 38.1 | 37.4 |
| 64.9 | 1.31928 | 35.5 | 34.8 | 70.1 | 1.35151 | 38.2 | 37.5 |
| **65.0** | 1.31989 | 35.6 | 34.9 | 70.2 | 1.35214 | 38.2 | 37.5 |
| 65.1 | 1.32050 | 35.6 | 34.95 | 70.3 | 1.35277 | 38.3 | 37.6 |
| 65.2 | 1.32111 | 35.7 | 35.0 | 70.4 | 1.35340 | 38.3 | 37.6 |
| 65.3 | 1.32172 | 35.7 | 35.05 | 70.5 | 1.35403 | 38.4 | 37.7 |
| 65.4 | 1.32233 | 35.8 | 35.1 | 70.6 | 1.35466 | 38.4 | 37.7 |
| 65.5 | 1.32294 | 35.8 | 35.15 | 70.7 | 1.35530 | 38.5 | 37.8 |
| 65.6 | 1.32355 | 35.9 | 35.2 | 70.8 | 1.35593 | 38.5 | 37.8 |
| 65.7 | 1.32417 | 35.9 | 35.25 | 70.9 | 1.35656 | 38.6 | 37.9 |
| 65.8 | 1.32478 | 36.0 | 35.3 | **71.0** | 1.35720 | 38.6 | 37.9 |
| 65.9 | 1.32539 | 36.0 | 35.35 | 71.1 | 1.35783 | 38.7 | 37.9 |
| **66.0** | 1.32601 | 36.1 | 35.4 | 71.2 | 1.35847 | 38.7 | 38.0 |
| 66.1 | 1.32662 | 36.1 | 35.5 | 71.3 | 1.35910 | 38.8 | 38.0 |
| 66.2 | 1.32724 | 36.2 | 35.5 | 71.4 | 1.35974 | 38.8 | 38.1 |
| 66.3 | 1.32785 | 36.2 | 35.6 | 71.5 | 1.36037 | 38.9 | 38.1 |
| 66.4 | 1.32847 | 36.3 | 35.6 | 71.6 | 1.36101 | 38.9 | 38.2 |
| 66.5 | 1.32908 | 36.3 | 35.7 | 71.7 | 1.36164 | 39.0 | 38.2 |
| 66.6 | 1.32970 | 36.4 | 35.7 | 71.8 | 1.36228 | 39.0 | 38.3 |
| 66.7 | 1.33031 | 36.4 | 35.8 | 71.9 | 1.36292 | 39.1 | 38.3 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| **72.0** | 1.36355 | 39.1 | 38.4 | 77.2 | 1.39726 | 41.7 | 40.9 |
| 72.1 | 1.36419 | 39.2 | 38.4 | 77.3 | 1.39792 | 41.8 | 41.0 |
| 72.2 | 1.36483 | 39.2 | 38.5 | 77.4 | 1.39858 | 41.8 | 41.0 |
| 72.3 | 1.36547 | 39.3 | 38.5 | 77.5 | 1.39924 | 41.9 | 41.1 |
| 72.4 | 1.36611 | 39.3 | 38.6 | 77.6 | 1.39990 | 41.9 | 41.1 |
| 72.5 | 1.36675 | 39.4 | 38.6 | 77.7 | 1.40056 | 42.0 | 41.2 |
| 72.6 | 1.36739 | 39.4 | 38.7 | 77.8 | 1.40122 | 42.0 | 41.2 |
| 72.7 | 1.36803 | 39.5 | 38.7 | 77.9 | 1.40188 | 42.1 | 41.3 |
| 72.8 | 1.36867 | 39.5 | 38.8 | **78.0** | 1.40254 | 42.1 | 41.3 |
| 72.9 | 1.36931 | 39.6 | 38.8 | 78.1 | 1.40321 | 42.2 | 41.4 |
| **73.0** | 1.36995 | 39.6 | 38.9 | 78.2 | 1.40387 | 42.2 | 41.4 |
| 73.1 | 1.37059 | 39.7 | 38.9 | 78.3 | 1.40453 | 42.3 | 41.5 |
| 73.2 | 1.37124 | 39.7 | 39.0 | 78.4 | 1.40520 | 42.3 | 41.5 |
| 73.3 | 1.37188 | 39.8 | 39.0 | 78.5 | 1.40586 | 42.4 | 41.6 |
| 73.4 | 1.37252 | 39.8 | 39.1 | 78.6 | 1.40652 | 42.4 | 41.6 |
| 73.5 | 1.37317 | 39.9 | 39.1 | 78.7 | 1.40719 | 42.5 | 41.7 |
| 73.6 | 1.37381 | 39.9 | 39.2 | 78.8 | 1.40785 | 42.5 | 41.7 |
| 73.7 | 1.37446 | 40.0 | 39.2 | 78.9 | 1.40852 | 42.6 | 41.8 |
| 73.8 | 1.37510 | 40.0 | 39.3 | **79.0** | 1.40918 | 42.6 | 41.8 |
| 73.9 | 1.37575 | 40.1 | 39.3 | 79.1 | 1.40985 | 42.7 | 41.9 |
| **74.0** | 1.37639 | 40.1 | 39.4 | 79.2 | 1.41052 | 42.7 | 41.9 |
| 74.1 | 1.37704 | 40.2 | 39.4 | 79.3 | 1.41118 | 42.8 | 42.0 |
| 74.2 | 1.37768 | 40.2 | 39.5 | 79.4 | 1.41185 | 42.8 | 42.0 |
| 74.3 | 1.37833 | 40.3 | 39.5 | 79.5 | 1.41252 | 42.9 | 42.1 |
| 74.4 | 1.37898 | 40.3 | 39.6 | 79.6 | 1.41318 | 42.9 | 42.1 |
| 74.5 | 1.37962 | 40.4 | 39.6 | 79.7 | 1.41385 | 43.0 | 42.1 |
| 74.6 | 1.38027 | 40.4 | 39.7 | 79.8 | 1.41452 | 43.0 | 42.2 |
| 74.7 | 1.38092 | 40.5 | 39.7 | 79.9 | 1.41519 | 43.1 | 42.2 |
| 74.8 | 1.38157 | 40.5 | 39.8 | **80.0** | 1.41586 | 43.1 | 42.3 |
| 74.9 | 1.38222 | 40.6 | 39.8 | 80.1 | 1.41653 | 43.2 | 42.3 |
| **75.0** | 1.38287 | 40.6 | 39.9 | 80.2 | 1.41720 | 43.2 | 42.4 |
| 75.1 | 1.38352 | 40.7 | 39.9 | 80.3 | 1.41787 | 43.2 | 42.4 |
| 75.2 | 1.38417 | 40.7 | 40.0 | 80.4 | 1.41854 | 43.3 | 42.5 |
| 75.3 | 1.38482 | 40.8 | 40.0 | 80.5 | 1.41921 | 43.3 | 42.5 |
| 75.4 | 1.38547 | 40.8 | 40.1 | 80.6 | 1.41989 | 43.4 | 42.6 |
| 75.5 | 1.38612 | 40.9 | 40.1 | 80.7 | 1.42056 | 43.45 | 42.6 |
| 75.6 | 1.38677 | 40.9 | 40.2 | 80.8 | 1.42123 | 43.5 | 42.7 |
| 75.7 | 1.38743 | 41.0 | 40.2 | 80.9 | 1.42190 | 43.55 | 42.7 |
| 75.8 | 1.38808 | 41.0 | 40.3 | **81.0** | 1.42258 | 43.6 | 42.8 |
| 75.9 | 1.38873 | 41.1 | 40.3 | 81.1 | 1.42325 | 43.65 | 42.8 |
| **76.0** | 1.38939 | 41.1 | 40.4 | 81.2 | 1.42393 | 43.7 | 42.9 |
| 76.1 | 1.39004 | 41.2 | 40.4 | 81.3 | 1.42460 | 43.7 | 42.9 |
| 76.2 | 1.39070 | 41.2 | 40.5 | 81.4 | 1.42528 | 43.8 | 43.0 |
| 76.3 | 1.39135 | 41.3 | 40.5 | 81.5 | 1.42595 | 43.8 | 43.0 |
| 76.4 | 1.39201 | 41.3 | 40.6 | 81.6 | 1.42663 | 43.9 | 43.1 |
| 76.5 | 1.39266 | 41.4 | 40.6 | 81.7 | 1.42731 | 43.9 | 43.1 |
| 76.6 | 1.39332 | 41.4 | 40.7 | 81.8 | 1.42798 | 44.0 | 43.2 |
| 76.7 | 1.39397 | 41.5 | 40.7 | 81.9 | 1.42866 | 44.0 | 43.2 |
| 76.8 | 1.39463 | 41.5 | 40.8 | **82.0** | 1.42934 | 44.1 | 43.2 |
| 76.9 | 1.39529 | 41.6 | 40.8 | 82.1 | 1.43002 | 44.1 | 43.3 |
| **77.0** | 1.39595 | 41.6 | 40.8 | 82.2 | 1.43070 | 44.2 | 43.3 |
| 77.1 | 1.39660 | 41.7 | 40.9 | 82.3 | 1.43137 | 44.2 | 43.4 |

TABLE 3. (*Continued.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 82.4 | 1.43205 | 44.3 | 43.4 | 87.6 | 1.46794 | 46.8 | 45.9 |
| 82.5 | 1.43273 | 44.3 | 43.5 | 87.7 | 1.46864 | 46.8 | 45.9 |
| 82.6 | 1.43341 | 44.4 | 43.5 | 87.8 | 1.46934 | 46.9 | 46.0 |
| 82.7 | 1.43409 | 44.4 | 43.6 | 87.9 | 1.47004 | 46.9 | 46.0 |
| 82.8 | 1.43478 | 44.5 | 43.6 | **88.0** | 1.47074 | 47.0 | 46.1 |
| 82.9 | 1.43546 | 44.5 | 43.7 | 88.1 | 1.47145 | 47.0 | 46.1 |
| **83.0** | 1.43614 | 44.6 | 43.7 | 88.2 | 1.47215 | 47.1 | 46.2 |
| 83.1 | 1.43682 | 44.6 | 43.8 | 88.3 | 1.47285 | 47.1 | 46.2 |
| 83.2 | 1.43750 | 44.7 | 43.8 | 88.4 | 1.47356 | 47.2 | 46.3 |
| 83.3 | 1.43819 | 44.7 | 43.9 | 88.5 | 1.47426 | 47.2 | 46.3 |
| 83.4 | 1.43887 | 44.8 | 43.9 | 88.6 | 1.47496 | 47.3 | 46.4 |
| 83.5 | 1.43955 | 44.8 | 44.0 | 88.7 | 1.47567 | 47.3 | 46.4 |
| 83.6 | 1.44024 | 44.9 | 44.0 | 88.8 | 1.47637 | 47.4 | 46.5 |
| 83.7 | 1.44092 | 44.9 | 44.1 | 88.9 | 1.47708 | 47.4 | 46.5 |
| 83.8 | 1.44161 | 45.0 | 44.1 | **89.0** | 1.47778 | 47.45 | 46.5 |
| 83.9 | 1.44229 | 45.0 | 44.2 | 89.1 | 1.47849 | 47.5 | 46.6 |
| **84.0** | 1.44298 | 45.1 | 44.2 | 89.2 | 1.47920 | 47.55 | 46.6 |
| 84.1 | 1.44367 | 45.1 | 44.2 | 89.3 | 1.47991 | 47.6 | 46.7 |
| 84.2 | 1.44435 | 45.15 | 44.3 | 89.4 | 1.48061 | 47.6 | 46.7 |
| 84.3 | 1.44504 | 45.2 | 44.3 | 89.5 | 1.48132 | 47.7 | 46.8 |
| 84.4 | 1.44573 | 45.25 | 44.4 | 89.6 | 1.48203 | 47.7 | 46.8 |
| 84.5 | 1.44641 | 45.3 | 44.4 | 89.7 | 1.48274 | 47.8 | 46.9 |
| 84.6 | 1.44710 | 45.35 | 44.5 | 89.8 | 1.48345 | 47.8 | 46.9 |
| 84.7 | 1.44779 | 45.4 | 44.5 | 89.9 | 1.48416 | 47.9 | 47.0 |
| 84.8 | 1.44848 | 45.4 | 44.6 | **90.0** | 1.48486 | 47.9 | 47.0 |
| 84.9 | 1.44917 | 45.5 | 44.6 | 90.1 | 1.48558 | 48.0 | 47.1 |
| **85.0** | 1.44986 | 45.5 | 44.7 | 90.2 | 1.48629 | 48.0 | 47.1 |
| 85.1 | 1.45055 | 45.6 | 44.7 | 90.3 | 1.48700 | 48.1 | 47.2 |
| 85.2 | 1.45124 | 45.6 | 44.8 | 90.4 | 1.48771 | 48.1 | 47.2 |
| 85.3 | 1.45193 | 45.7 | 44.8 | 90.5 | 1.48842 | 48.2 | 47.2 |
| 85.4 | 1.45262 | 45.7 | 44.9 | 90.6 | 1.48913 | 48.2 | 47.3 |
| 85.5 | 1.45331 | 45.8 | 44.9 | 90.7 | 1.48985 | 48.3 | 47.3 |
| 85.6 | 1.45401 | 45.8 | 45.0 | 90.8 | 1.49056 | 48.3 | 47.4 |
| 85.7 | 1.45470 | 45.9 | 45.0 | 90.9 | 1.49127 | 48.35 | 47.4 |
| 85.8 | 1.45539 | 45.9 | 45.0 | **91.0** | 1.49199 | 48.4 | 47.5 |
| 85.9 | 1.45609 | 46.0 | 45.1 | 91.1 | 1.49270 | 48.45 | 47.5 |
| **86.0** | 1.45678 | 46.0 | 45.1 | 91.2 | 1.49342 | 48.5 | 47.6 |
| 86.1 | 1.45748 | 46.1 | 45.2 | 91.3 | 1.49413 | 48.5 | 47.6 |
| 86.2 | 1.45817 | 46.1 | 45.2 | 91.4 | 1.49485 | 48.6 | 47.7 |
| 86.3 | 1.45887 | 46.2 | 45.3 | 91.5 | 1.49556 | 48.6 | 47.7 |
| 86.4 | 1.45956 | 46.2 | 45.3 | 91.6 | 1.49628 | 48.7 | 47.8 |
| 86.5 | 1.46026 | 46.3 | 45.4 | 91.7 | 1.49700 | 48.7 | 47.8 |
| 86.6 | 1.46095 | 46.3 | 45.4 | 91.8 | 1.49771 | 48.8 | 47.8 |
| 86.7 | 1.46165 | 46.35 | 45.5 | 91.9 | 1.49843 | 48.8 | 47.9 |
| 86.8 | 1.46235 | 46.4 | 45.5 | **92.0** | 1.49915 | 48.9 | 47.9 |
| 86.9 | 1.46304 | 46.45 | 45.6 | 92.1 | 1.49987 | 48.9 | 48.0 |
| **87.0** | 1.46374 | 46.5 | 45.6 | 92.2 | 1.50058 | 49.0 | 48.0 |
| 87.1 | 1.46444 | 46.55 | 45.7 | 92.3 | 1.50130 | 49.0 | 48.1 |
| 87.2 | 1.46514 | 46.6 | 45.7 | 92.4 | 1.50202 | 49.05 | 48.1 |
| 87.3 | 1.46584 | 46.65 | 45.8 | 92.5 | 1.50274 | 49.1 | 48.2 |
| 87.4 | 1.46654 | 46.7 | 45.8 | 92.6 | 1.50346 | 49.15 | 48.2 |
| 87.5 | 1.46724 | 46.7 | 45.8 | 92.7 | 1.50419 | 49.2 | 48.3 |

TABLE 3. (*Concluded.*)

| Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | | Per cent sucrose by weight or degrees Brix. | Specific gravity. | Degrees Baumé. | |
|---|---|---|---|---|---|---|---|
| | | New. | Old. | | | New. | Old. |
| 92.8 | 1.50491 | 49.25 | 48.3 | **94.0** | 1.51359 | 49.8 | 48.8 |
| 92.9 | 1.50563 | 49.3 | 48.3 | 94.1 | 1.51431 | 49.85 | 48.9 |
| **93.0** | 1.50635 | 49.3 | 48.4 | 94.2 | 1.51504 | 49.9 | 48.9 |
| 93.1 | 1.50707 | 49.4 | 48.4 | 94.3 | 1.51577 | 49.9 | 49.0 |
| 93.2 | 1.50779 | 49.4 | 48.5 | 94.4 | 1.51649 | 50.0 | 49.0 |
| 93.3 | 1.50852 | 49.5 | 48.5 | 94.5 | 1.51722 | 50.0 | 49.1 |
| 93.4 | 1.50924 | 49.5 | 48.6 | 94.6 | 1.51795 | 50.1 | 49.1 |
| 93.5 | 1.50996 | 49.6 | 48.6 | 94.7 | 1.51868 | 50.1 | 49.2 |
| 93.6 | 1.51069 | 49.6 | 48.7 | 94.8 | 1.51941 | 50.2 | 49.2 |
| 93.7 | 1.51141 | 49.7 | 48.7 | 94.9 | 1.52014 | 50.2 | 49.3 |
| 93.8 | 1.51214 | 49.7 | 48.8 | **95.0** | 1.52087 | 50.3 | 49.3 |
| 93.9 | 1.51286 | 49.8 | 48.8 | | | | |

## TABLE * 4.

### Table for Correcting Readings of Brix Hydrometers at Different Temperatures to 17.5°C.

| Temperature. Degrees Centigrade. | Degrees Brix of solution. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 50 | 60 | 70 | 75 |
| | Corrections to be subtracted from degrees Brix. | | | | | | | | | | | | |
| 0° | 0.17 | 0.30 | 0.41 | 0.52 | 0.62 | 0.72 | 0.82 | 0.92 | 0.98 | 1.11 | 1.22 | 1.25 | 1.29 |
| 5 | 0.23 | 0.30 | 0.37 | 0.44 | 0.52 | 0.59 | 0.65 | 0.72 | 0.75 | 0.80 | 0.88 | 0.91 | 0.94 |
| 10 | 0.20 | 0.26 | 0.29 | 0.33 | 0.36 | 0.39 | 0.42 | 0.45 | 0.48 | 0.50 | 0.54 | 0.58 | 0.61 |
| 11 | 0.18 | 0.23 | 0.26 | 0.28 | 0.31 | 0.34 | 0.36 | 0.39 | 0.41 | 0.43 | 0.47 | 0.50 | 0.53 |
| 12 | 0.16 | 0.20 | 0.22 | 0.24 | 0.26 | 0.29 | 0.31 | 0.33 | 0.34 | 0.36 | 0.40 | 0.42 | 0.46 |
| 13 | 0.14 | 0.18 | 0.19 | 0.21 | 0.22 | 0.24 | 0.26 | 0.27 | 0.28 | 0.29 | 0.33 | 0.35 | 0.39 |
| 14 | 0.12 | 0.15 | 0.16 | 0.17 | 0.18 | 0.19 | 0.21 | 0.22 | 0.22 | 0.23 | 0.26 | 0.28 | 0.32 |
| 15 | 0.09 | 0.11 | 0.12 | 0.14 | 0.14 | 0.15 | 0.16 | 0.17 | 0.16 | 0.17 | 0.19 | 0.21 | 0.25 |
| 16 | 0.06 | 0.07 | 0.08 | 0.09 | 0.10 | 0.10 | 0.11 | 0.12 | 0.12 | 0.12 | 0.14 | 0.16 | 0.18 |
| 17 | 0.02 | 0.02 | 0.03 | 0.03 | 0.03 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 | 0.06 |
| | Corrections to be added to degrees Brix. | | | | | | | | | | | | |
| 18 | 0.02 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 |
| 19 | 0.06 | 0.08 | 0.08 | 0.09 | 0.09 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.08 | 0.06 |
| 20 | 0.11 | 0.14 | 0.15 | 0.17 | 0.17 | 0.18 | 0.18 | 0.18 | 0.19 | 0.19 | 0.18 | 0.15 | 0.11 |
| 21 | 0.16 | 0.20 | 0.22 | 0.24 | 0.24 | 0.25 | 0.25 | 0.25 | 0.26 | 0.26 | 0.25 | 0.22 | 0.18 |
| 22 | 0.21 | 0.26 | 0.29 | 0.31 | 0.31 | 0.32 | 0.32 | 0.32 | 0.33 | 0.34 | 0.32 | 0.29 | 0.25 |
| 23 | 0.27 | 0.32 | 0.35 | 0.37 | 0.38 | 0.39 | 0.39 | 0.39 | 0.40 | 0.42 | 0.39 | 0.36 | 0.33 |
| 24 | 0.32 | 0.38 | 0.41 | 0.43 | 0.44 | 0.46 | 0.46 | 0.47 | 0.47 | 0.50 | 0.46 | 0.43 | 0.40 |
| 25 | 0.37 | 0.44 | 0.47 | 0.49 | 0.51 | 0.53 | 0.54 | 0.55 | 0.55 | 0.58 | 0.54 | 0.51 | 0.48 |
| 26 | 0.43 | 0.50 | 0.54 | 0.56 | 0.58 | 0.60 | 0.61 | 0.62 | 0.62 | 0.66 | 0.62 | 0.58 | 0.55 |
| 27 | 0.49 | 0.57 | 0.61 | 0.63 | 0.65 | 0.68 | 0.68 | 0.69 | 0.70 | 0.74 | 0.70 | 0.65 | 0.62 |
| 28 | 0.56 | 0.64 | 0.68 | 0.70 | 0.72 | 0.76 | 0.76 | 0.78 | 0.78 | 0.82 | 0.78 | 0.72 | 0.70 |
| 29 | 0.63 | 0.71 | 0.75 | 0.78 | 0.79 | 0.84 | 0.84 | 0.86 | 0.86 | 0.90 | 0.86 | 0.80 | 0.78 |
| 30 | 0.70 | 0.78 | 0.82 | 0.87 | 0.87 | 0.92 | 0.92 | 0.94 | 0.94 | 0.98 | 0.94 | 0.88 | 0.86 |
| 35 | 1.10 | 1.17 | 1.22 | 1.24 | 1.30 | 1.32 | 1.33 | 1.35 | 1.36 | 1.39 | 1.34 | 1.27 | 1.25 |
| 40 | 1.50 | 1.61 | 1.67 | 1.71 | 1.73 | 1.79 | 1.79 | 1.80 | 1.82 | 1.83 | 1.78 | 1.69 | 1.65 |
| 50 | .... | 2.65 | 2.71 | 2.74 | 2.78 | 2.80 | 2.80 | 2.80 | 2.80 | 2.79 | 2.70 | 2.56 | 2.51 |
| 60 | .... | 3.87 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.90 | 3.82 | 3.70 | 3.43 | 3.41 |
| 70 | .... | 5.17 | 5.18 | 5.20 | 5.14 | 5.13 | 5.10 | 5.08 | 5.06 | 4.90 | 4.72 | 4.47 | 4.35 |
| 80 | .... | .... | 6.62 | 6.59 | 6.54 | 6.46 | 6.38 | 6.30 | 6.26 | 6.06 | 5.82 | 5.50 | 5.33 |
| 90 | .... | .... | 8.26 | 8.16 | 8.06 | 7.97 | 7.83 | 7.71 | 7.58 | 7.30 | 6.96 | 6.58 | 6.37 |
| 100 | .... | .... | 10.01 | 9.87 | 9.72 | 9.56 | 9.39 | 9.21 | 9.03 | 8.64 | 8.22 | 7.76 | 7.42 |

* *See* "Handbook," page 31.

## TABLE* 5.

### MAIN'S TABLE FOR DETERMINING WATER IN SUGAR SOLUTIONS BY MEANS OF THE ABBE REFRACTOMETER.

| Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. |
|---|---|---|---|---|---|---|---|
| | Per cent. | | Per cent. | | Per cent. | | Per cent. |
| **1.3330** | 100 | 1.3397 | 95.2 | 1.3469 | 90.4 | 1.3545 | 85.6 |
| 1.3331 | 99.9 | 1.3399 | 95.1 | 1.3471 | 90.3 | 1.3546 | 85.5 |
| 1.3333 | 99.8 | **1.3400** | 95 | 1.3472 | 90.2 | 1.3548 | 85.4 |
| 1.3334 | 99.7 | 1.3402 | 94.9 | 1.3474 | 90.1 | 1.3549 | 85.3 |
| 1.3336 | 99.6 | 1.3403 | 94.8 | 1.3475 | 90 | 1.3551 | 85.2 |
| 1.3337 | 99.5 | 1.3405 | 94.7 | 1.3477 | 89.9 | 1.3552 | 85.1 |
| 1.3338 | 99.4 | 1.3406 | 94.6 | 1.3478 | 89.8 | 1.3554 | 85 |
| 1.3340 | 99.3 | 1.3408 | 94.5 | 1.3480 | 89.7 | 1.3556 | 84.9 |
| 1.3341 | 99.2 | 1.3409 | 94.4 | 1.3481 | 89.6 | 1.3557 | 84.8 |
| 1.3343 | 99.1 | 1.3411 | 94.3 | 1.3483 | 89.5 | 1.3559 | 84.7 |
| 1.3344 | 99 | 1.3412 | 94.2 | 1.3484 | 89.4 | 1.3561 | 84.6 |
| 1.3345 | 98.9 | 1.3414 | 94.1 | 1.3486 | 89.3 | 1.3562 | 84.5 |
| 1.3347 | 98.8 | 1.3415 | 94 | 1.3488 | 89.2 | 1.3564 | 84.4 |
| 1.3348 | 98.7 | 1.3417 | 93.9 | 1.3489 | 89.1 | 1.3566 | 84.3 |
| 1.3350 | 98.6 | 1.3418 | 93.8 | 1.3491 | 89 | 1.3567 | 84.2 |
| 1.3351 | 98.5 | 1.3420 | 93.7 | 1.3492 | 88.9 | 1.3569 | 84.1 |
| 1.3352 | 98.4 | 1.3421 | 93.6 | 1.3494 | 88.8 | 1.3571 | 84 |
| 1.3354 | 98.3 | 1.3423 | 93.5 | 1.3496 | 88.7 | 1.3572 | 83.9 |
| 1.3355 | 98.2 | 1.3424 | 93.4 | 1.3497 | 88.6 | 1.3574 | 83.8 |
| 1.3357 | 98.1 | 1.3426 | 93.3 | 1.3499 | 88.5 | 1.3576 | 83.7 |
| 1.3358 | 98 | 1.3427 | 93.2 | **1.3500** | 88.4 | 1.3577 | 83.6 |
| 1.3359 | 97.9 | 1.3429 | 93.1 | 1.3502 | 88.3 | 1.3579 | 83.5 |
| 1.3361 | 97.8 | 1.3430 | 93 | 1.3503 | 88.2 | 1.3581 | 83.4 |
| 1.3362 | 97.7 | 1.3432 | 92.9 | 1.3505 | 88.1 | 1.3582 | 83.3 |
| 1.3364 | 97.6 | 1.3433 | 92.8 | 1.3507 | 88 | 1.3584 | 83.2 |
| 1.3365 | 97.5 | 1.3435 | 92.7 | 1.3508 | 87.9 | 1.3586 | 83.1 |
| 1.3366 | 97.4 | 1.3436 | 92.6 | 1.3510 | 87.8 | 1.3587 | 83 |
| 1.3368 | 97.3 | 1.3438 | 92.5 | 1.3511 | 87.7 | 1.3589 | 82.9 |
| 1.3369 | 97.2 | 1.3439 | 92.4 | 1.3513 | 87.6 | 1.3591 | 82.8 |
| 1.3371 | 97.1 | 1.3441 | 92.3 | 1.3515 | 87.5 | 1.3592 | 82.7 |
| 1.3372 | 97 | 1.3442 | 92.2 | 1.3516 | 87.4 | 1.3594 | 82.6 |
| 1.3373 | 96.9 | 1.3444 | 92.1 | 1.3518 | 87.3 | 1.3596 | 82.5 |
| 1.3375 | 96.8 | 1.3445 | 92 | 1.3519 | 87.2 | 1.3597 | 82.4 |
| 1.3376 | 96.7 | 1.3447 | 91.9 | 1.3521 | 87.1 | 1.3599 | 82.3 |
| 1.3378 | 96.6 | 1.3448 | 91.8 | 1.3522 | 87 | **1.3600** | 82.2 |
| 1.3379 | 96.5 | 1.3450 | 91.7 | 1.3524 | 86.9 | 1.3602 | 82.1 |
| 1.3380 | 96.4 | 1.3451 | 91.6 | 1.3526 | 86.8 | 1.3604 | 82 |
| 1.3382 | 96.3 | 1.3453 | 91.5 | 1.3527 | 86.7 | 1.3605 | 81.9 |
| 1.3383 | 96.2 | 1.3454 | 91.4 | 1.3529 | 86.6 | 1.3607 | 81.8 |
| 1.3385 | 96.1 | 1.3456 | 91.3 | 1.3530 | 86.5 | 1.3609 | 81.7 |
| 1.3386 | 96 | 1.3457 | 91.2 | 1.3532 | 86.4 | 1.3610 | 81.6 |
| 1.3387 | 95.9 | 1.3459 | 91.1 | 1.3533 | 86.3 | 1.3612 | 81.5 |
| 1.3389 | 95.8 | 1.3460 | 91 | 1.3535 | 86.2 | 1.3614 | 81.4 |
| 1.3390 | 95.7 | 1.3462 | 90.9 | 1.3537 | 86.1 | 1.3615 | 81.3 |
| 1.3392 | 95.6 | 1.3463 | 90.8 | 1.3538 | 86 | 1.3617 | 81.2 |
| 1.3393 | 95.5 | 1.3465 | 90.7 | 1.3540 | 85.9 | 1.3619 | 81.1 |
| 1.3394 | 95.4 | 1.3466 | 90.6 | 1.3541 | 85.8 | 1.3620 | 81 |
| 1.3396 | 95.3 | 1.3468 | 90.5 | 1.3543 | 85.7 | 1.3622 | 80.9 |

* *See* "Handbook," page 64.

TABLE 5. (*Continued.*)

| Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. |
|---|---|---|---|---|---|---|---|
| | Per cent. | | Per cent. | | Per cent. | | Per cent. |
| 1.3624 | 80.8 | 1.3709 | 75.7 | 1.3799 | 70.6 | 1.3893 | 65.5 |
| 1.3625 | 80.7 | 1.3711 | 75.6 | **1.3801** | 70.5 | 1.3895 | 65.4 |
| 1.3627 | 80.6 | 1.3713 | 75.5 | 1.3803 | 70.4 | 1.3896 | 65.3 |
| 1.3629 | 80.5 | 1.3714 | 75.4 | 1.3805 | 70.3 | 1.3898 | 65.2 |
| 1.3630 | 80.4 | 1.3716 | 75.3 | 1.3806 | 70.2 | **1.3900** | 65.1 |
| 1.3632 | 80.3 | 1.3718 | 75.2 | 1.3808 | 70.1 | 1.3902 | 65 |
| 1.3634 | 80.2 | 1.3719 | 75.1 | 1.3810 | 70 | 1.3904 | 64.9 |
| 1.3635 | 80.1 | 1.3721 | 75 | 1.3812 | 69.9 | 1.3906 | 64.8 |
| 1.3637 | 80 | 1.3723 | 74.9 | 1.3814 | 69.8 | 1.3908 | 64.7 |
| 1.3639 | 79.9 | 1.3725 | 74.8 | 1.3816 | 69.7 | 1.3910 | 64.6 |
| 1.3640 | 79.8 | 1.3726 | 74.7 | 1.3817 | 69.6 | 1.3912 | 64.5 |
| 1.3642 | 79.7 | 1.3728 | 74.6 | 1.3819 | 69.5 | 1.3913 | 64.4 |
| 1.3644 | 79.6 | 1.3730 | 74.5 | 1.3821 | 69.4 | 1.3915 | 64.3 |
| 1.3645 | 79.5 | 1.3732 | 74.4 | 1.3823 | 69.3 | 1.3917 | 64.2 |
| 1.3647 | 79.4 | 1.3733 | 74.3 | 1.3825 | 69.2 | 1.3919 | 64.1 |
| 1.3649 | 79.3 | 1.3735 | 74.2 | 1.3827 | 69.1 | 1.3921 | 64 |
| 1.3650 | 79.2 | 1.3737 | 74.1 | 1.3828 | 69 | 1.3923 | 63.9 |
| 1.3652 | 79.1 | 1.3739 | 74 | 1.3830 | 68.9 | 1 3925 | 63.8 |
| 1.3654 | 79 | 1.3741 | 73.9 | 1.3832 | 68.8 | 1.3927 | 63.7 |
| 1.3655 | 78.9 | 1.3742 | 73.8 | 1.3834 | 68.7 | 1.3929 | 63.6 |
| 1.3657 | 78.8 | 1.3744 | 73.7 | 1.3836 | 68.6 | 1.3931 | 63.5 |
| 1.3659 | 78.7 | 1.3746 | 73.6 | 1.3838 | 68.5 | 1.3932 | 63.4 |
| 1.3661 | 78.6 | 1.3748 | 73.5 | 1.3839 | 68.4 | 1.3934 | 63.3 |
| 1.3662 | 78.5 | 1.3749 | 73.4 | 1.3841 | 68.3 | 1.3936 | 63.2 |
| 1.3664 | 78.4 | 1.3751 | 73.3 | 1.3843 | 68.2 | 1.3938 | 63.1 |
| 1.3666 | 78.3 | 1.3753 | 73.2 | 1.3845 | 68.1 | 1.3940 | 63 |
| 1.3667 | 78.2 | 1.3755 | 73.1 | 1.3847 | 68 | 1.3942 | 62.9 |
| 1.3669 | 78.1 | 1.3757 | 73 | 1.3849 | 67.9 | 1.3944 | 62.8 |
| 1.3671 | 78 | 1.3758 | 72.9 | 1.3850 | 67.8 | 1.3946 | 62.7 |
| 1.3672 | 77.9 | 1.3760 | 72.8 | 1.3852 | 67.7 | 1.3948 | 62.6 |
| 1.3674 | 77.8 | 1.3762 | 72.7 | 1.3854 | 67.6 | 1.3950 | 62.5 |
| 1.3676 | 77.7 | 1.3764 | 72.6 | 1.3856 | 67.5 | 1.3951 | 62.4 |
| 1.3677 | 77.6 | 1.3766 | 72.5 | 1.3858 | 67.4 | 1.3953 | 62.3 |
| 1.3679 | 77.5 | 1.3767 | 72.4 | 1.3860 | 67.3 | 1.3955 | 62.2 |
| 1.3681 | 77.4 | 1.3769 | 72.3 | 1.3862 | 67.2 | 1.3957 | 62.1 |
| 1.3682 | 77.3 | 1.3771 | 72.2 | 1.3863 | 67.1 | 1.3959 | 62 |
| 1.3684 | 77.2 | 1.3773 | 72.1 | 1.3865 | 67 | 1.3961 | 61.9 |
| 1.3686 | 77.1 | 1.3774 | 72 | 1.3867 | 66.9 | 1.3963 | 61.8 |
| 1.3687 | 77 | 1.3776 | 71.9 | 1.3869 | 66.8 | 1.3965 | 61.7 |
| 1.3689 | 76.9 | 1.3778 | 71.8 | 1.3871 | 66.7 | 1.3967 | 61.6 |
| 1.3691 | 76.8 | 1.3780 | 71.7 | 1.3873 | 66.6 | 1.3969 | 61.5 |
| 1.3692 | 76.7 | 1.3782 | 71.6 | 1.3874 | 66.5 | 1.3970 | 61.4 |
| 1.3694 | 76.6 | 1.3783 | 71.5 | 1.3876 | 66.4 | 1.3972 | 61.3 |
| 1.3696 | 76.5 | 1.3785 | 71.4 | 1.3878 | 66.3 | 1.3974 | 61.2 |
| 1.3697 | 76.4 | 1.3787 | 71.3 | 1.3880 | 66.2 | 1.3976 | 61.1 |
| 1.3699 | 76.3 | 1.3789 | 71.2 | 1.3882 | 66.1 | 1.3978 | 61 |
| **1.3701** | 76.2 | 1.3790 | 71.1 | 1.3884 | 66 | 1.3980 | 60.9 |
| 1.3703 | 76.1 | 1.3792 | 71 | 1.3885 | 65.9 | 1.3982 | 60.8 |
| 1.3704 | 76 | 1.3794 | 70.9 | 1.3887 | 65.8 | 1.3984 | 60.7 |
| 1.3706 | 75.9 | 1.3796 | 70.8 | 1.3889 | 65.7 | 1.3986 | 60.6 |
| 1.3708 | 75.8 | 1.3798 | 70.7 | 1.3891 | 65.6 | 1.3988 | 60.5 |

TABLE 5. (*Continued.*)

| Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. |
|---|---|---|---|---|---|---|---|
| | Per cent. | | Per cent. | | Per cent. | | Per cent. |
| 1.3989 | 60.4 | 1.4089 | 55.3 | 1.4197 | 50.2 | 1.4302 | 45.1 |
| 1.3991 | 60.3 | 1.4091 | 55.2 | 1.4199 | 50.1 | 1.4304 | 45 |
| 1.3993 | 60.2 | 1.4093 | 55.1 | **1.4201** | 50 | 1.4306 | 44.9 |
| 1.3995 | 60.1 | 1.4095 | 55 | 1.4203 | 49.9 | 1.4309 | 44.8 |
| 1.3997 | 60 | 1.4097 | 54.9 | 1.4205 | 49.8 | 1.4311 | 44.7 |
| 1.3999 | 59.9 | 1.4099 | 54.8 | 1.4207 | 49.7 | 1.4313 | 44.6 |
| **1.4001** | 59.8 | **1.4101** | 54.7 | 1.4209 | 49.6 | 1.4316 | 44.5 |
| 1.4003 | 59.7 | 1.4103 | 54.6 | 1.4211 | 49.5 | 1.4318 | 44.4 |
| 1.4005 | 59.6 | 1.4106 | 54.5 | 1.4213 | 49.4 | 1.4320 | 44.3 |
| 1.4007 | 59.5 | 1.4108 | 54.4 | 1.4215 | 49.3 | 1.4322 | 44.2 |
| 1.4009 | 59.4 | 1.4110 | 54.3 | 1.4217 | 49.2 | 1.4325 | 44.1 |
| 1.4011 | 59.3 | 1.4112 | 54.2 | 1.4220 | 49.1 | 1.4327 | 44 |
| 1.4013 | 59.2 | 1.4114 | 54.1 | 1.4222 | 49 | 1.4329 | 43.9 |
| 1.4015 | 59.1 | 1.4116 | 54 | 1.4224 | 48.9 | 1.4332 | 43.8 |
| 1.4017 | 59 | 1.4118 | 53.9 | 1.4226 | 48.8 | 1.4334 | 43.7 |
| 1.4019 | 58.9 | 1.4120 | 53.8 | 1.4228 | 48.7 | 1.4336 | 43.6 |
| 1.4021 | 58.8 | 1.4123 | 53.7 | 1.4230 | 48.6 | 1.4339 | 43.5 |
| 1.4022 | 58.7 | 1.4125 | 53.6 | 1.4232 | 48.5 | 1.4341 | 43.4 |
| 1.4024 | 58.6 | 1.4127 | 53.5 | 1.4234 | 48.4 | 1.4343 | 43.3 |
| 1.4026 | 58.5 | 1.4129 | 53.4 | 1.4236 | 48.3 | 1.4345 | 43.2 |
| 1.4028 | 58.4 | 1.4131 | 53.3 | 1.4238 | 48.2 | 1.4348 | 43.1 |
| 1.4030 | 58.3 | 1.4133 | 53.2 | 1.4240 | 48.1 | 1.4350 | 43 |
| 1.4032 | 58.2 | 1.4135 | 53.1 | 1.4242 | 48 | 1.4352 | 42.9 |
| 1.4034 | 58.1 | 1.4137 | 53 | 1.4244 | 47.9 | 1.4355 | 42.8 |
| 1.4036 | 58 | 1.4140 | 52.9 | 1.4246 | 47.8 | 1.4357 | 42.7 |
| 1.4038 | 57.9 | 1.4142 | 52.8 | 1.4248 | 47.7 | 1.4359 | 42.6 |
| 1.4040 | 57.8 | 1.4144 | 52.7 | 1.4250 | 47.6 | 1.4362 | 42.5 |
| 1.4042 | 57.7 | 1.4146 | 52.6 | 1.4253 | 47.5 | 1.4364 | 42.4 |
| 1.4044 | 57.6 | 1.4148 | 52.5 | 1.4255 | 47.4 | 1.4366 | 42.3 |
| 1.4046 | 57.5 | 1.4150 | 52.4 | 1.4257 | 47.3 | 1.4368 | 42.2 |
| 1.4048 | 57.4 | 1.4152 | 52.3 | 1.4259 | 47.2 | 1.4371 | 42.1 |
| 1.4050 | 57.3 | 1.4154 | 52.2 | 1.4261 | 47.1 | 1.4373 | 42 |
| 1.4052 | 57.2 | 1.4156 | 52.1 | 1.4263 | 47 | 1.4375 | 41.9 |
| 1.4054 | 57.1 | 1.4159 | 52 | 1.4265 | 46.9 | 1.4378 | 41.8 |
| 1.4056 | 57 | 1.4161 | 51.9 | 1.4267 | 46.8 | 1.4380 | 41.7 |
| 1.4058 | 56.9 | 1.4163 | 51.8 | 1.4269 | 46.7 | 1.4382 | 41.6 |
| 1.4060 | 56.8 | 1.4165 | 51.7 | 1.4271 | 46.6 | 1.4385 | 41.5 |
| 1.4062 | 56.7 | 1.4167 | 51.6 | 1.4273 | 46.5 | 1.4387 | 41.4 |
| 1.4064 | 56.6 | 1.4169 | 51.5 | 1.4275 | 46.4 | 1.4389 | 41.3 |
| 1.4066 | 56.5 | 1.4171 | 51.4 | 1.4277 | 46.3 | 1.4391 | 41.2 |
| 1.4068 | 56.4 | 1.4173 | 51.3 | 1.4279 | 46.2 | 1.4394 | 41.1 |
| 1.4070 | 56.3 | 1.4176 | 51.2 | 1.4281 | 46.1 | 1.4396 | 41 |
| 1.4071 | 56.2 | 1.4178 | 51.1 | 1.4283 | 46 | 1.4398 | 40.9 |
| 1.4073 | 56.1 | 1.4180 | 51 | 1.4285 | 45.9 | **1.4401** | 40.8 |
| 1.4075 | 56 | 1.4182 | 50.9 | 1.4288 | 45.8 | 1.4403 | 40.7 |
| 1.4077 | 55.9 | 1.4184 | 50.8 | 1.4290 | 45.7 | 1.4405 | 40.6 |
| 1.4079 | 55.8 | 1.4186 | 50.7 | 1.4292 | 45.6 | 1.4408 | 40.5 |
| 1.4081 | 55.7 | 1.4188 | 50.6 | 1.4294 | 45.5 | 1.4410 | 40.4 |
| 1.4083 | 55.6 | 1.4190 | 50.5 | 1.4296 | 45.4 | 1.4412 | 40.3 |
| 1.4085 | 55.5 | 1.4193 | 50.4 | 1.4298 | 45.3 | 1.4414 | 40.2 |
| 1.4087 | 55.4 | 1.4195 | 50.3 | **1.4300** | 45.2 | 1.4417 | 40.1 |

TABLE 5. (*Continued.*)

| Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. |
|---|---|---|---|---|---|---|---|
| | Per cent. | | Per cent. | | Per cent. | | Per cent. |
| 1.4419 | 40 | 1.4537 | 34.9 | 1.4656 | 29.8 | 1.4782 | 24.7 |
| 1.4421 | 39.9 | 1.4540 | 34.8 | 1.4658 | 29.7 | 1.4784 | 24.6 |
| 1.4424 | 39.8 | 1.4542 | 34.7 | 1.4661 | 29.6 | 1.4787 | 24.5 |
| 1.4426 | 39.7 | 1.4544 | 34.6 | 1.4663 | 29.5 | 1.4789 | 24.4 |
| 1.4428 | 39.6 | 1.4547 | 34.5 | 1.4666 | 29.4 | 1.4792 | 24.3 |
| 1.4431 | 39.5 | 1.4549 | 34.4 | 1.4668 | 29.3 | 1.4794 | 24.2 |
| 1.4433 | 39.4 | 1.4551 | 34.3 | 1.4671 | 29.2 | 1.4797 | 24.1 |
| 1.4435 | 39.3 | 1.4554 | 34.2 | 1.4673 | 29.1 | 1.4799 | 24 |
| 1.4438 | 39.2 | 1.4556 | 34.1 | 1.4676 | 29 | **1.4802** | 23.9 |
| 1.4440 | 39.1 | 1.4558 | 34 | 1.4678 | 28.9 | 1.4804 | 23.8 |
| 1.4442 | 39 | 1.4561 | 33.9 | 1.4681 | 28.8 | 1.4807 | 23.7 |
| 1.4445 | 38.9 | 1.4563 | 33.8 | 1.4683 | 28.7 | 1.4810 | 23.6 |
| 1.4447 | 38.8 | 1.4565 | 33.7 | 1.4685 | 28.6 | 1.4812 | 23.5 |
| 1.4449 | 38.7 | 1.4567 | 33.6 | 1.4688 | 28.5 | 1.4815 | 23.4 |
| 1.4451 | 38.6 | 1.4570 | 33.5 | 1.4690 | 28.4 | 1.4817 | 23.3 |
| 1.4454 | 38.5 | 1.4572 | 33.4 | 1.4693 | 28.3 | 1.4820 | 23.2 |
| 1.4456 | 38.4 | 1.4574 | 33.3 | 1.4695 | 28.2 | 1.4822 | 23.1 |
| 1.4458 | 38.3 | 1.4577 | 33.2 | 1.4698 | 28.1 | 1.4825 | 23 |
| 1.4461 | 38.2 | 1.4579 | 33.1 | **1.4700** | 28 | 1.4827 | 22.9 |
| 1.4463 | 38.1 | 1.4581 | 33 | 1.4703 | 27.9 | 1.4830 | 22.8 |
| 1.4465 | 38 | 1.4584 | 32.9 | 1.4705 | 27.8 | 1.4832 | 22.7 |
| 1.4468 | 37.9 | 1.4586 | 32.8 | 1.4708 | 27.7 | 1.4835 | 22.6 |
| 1.4470 | 37.8 | 1.4588 | 32.7 | 1.4710 | 27.6 | 1.4838 | 22.5 |
| 1.4472 | 37.7 | 1.4591 | 32.6 | 1.4713 | 27.5 | 1.4840 | 22.4 |
| 1.4475 | 37.6 | 1.4593 | 32.5 | 1.4715 | 27.4 | 1.4843 | 22.3 |
| 1.4477 | 37.5 | 1.4595 | 32.4 | 1.4717 | 27.3 | 1.4845 | 22.2 |
| 1.4479 | 37.4 | 1.4598 | 32.3 | 1.4720 | 27.2 | 1.4848 | 22.1 |
| 1.4482 | 37.3 | **1.4600** | 32.2 | 1.4722 | 27.1 | 1.4850 | 22 |
| 1.4484 | 57.2 | 1.4602 | 32.1 | 1.4725 | 27 | 1.4853 | 21.9 |
| 1.4486 | 37.1 | 1.4605 | 32 | 1.4727 | 26.9 | 1.4855 | 21.8 |
| 1.4489 | 37 | 1.4607 | 31.9 | 1.4730 | 26.8 | 1.4858 | 21.7 |
| 1.4491 | 36.9 | 1.4609 | 31.8 | 1.4732 | 26.7 | 1.4860 | 21.6 |
| 1.4493 | 36.8 | 1.4612 | 31.7 | 1.4735 | 26.6 | 1.4863 | 21.5 |
| 1.4496 | 36.7 | 1.4614 | 31.6 | 1.4737 | 26.5 | 1.4865 | 21.4 |
| 1.4498 | 36.6 | 1.4616 | 31.5 | 1.4740 | 26.4 | 1.4868 | 21.3 |
| **1.4500** | 36.5 | 1.4619 | 31.4 | 1.4742 | 26.3 | 1.4871 | 21.2 |
| 1.4503 | 36.4 | 1.4621 | 31.3 | 1.4744 | 26.2 | 1.4873 | 21.1 |
| 1.4505 | 36.3 | 1.4623 | 31.2 | 1.4747 | 26.1 | 1.4876 | 21 |
| 1.4507 | 36.2 | 1.4625 | 31.1 | 1.4749 | 26 | 1.4878 | 20.9 |
| 1.4509 | 36.1 | 1.4628 | 31 | 1.4752 | 25.9 | 1.4881 | 20.8 |
| 1.4512 | 36 | 1.4630 | 30.9 | 1.4754 | 25.8 | 1.4883 | 20.7 |
| 1.4514 | 35.9 | 1.4632 | 30.8 | 1.4757 | 25.7 | 1.4886 | 20.6 |
| 1.4516 | 35.8 | 1.4635 | 30.7 | 1.4759 | 25.6 | 1.4888 | 20.5 |
| 1.4519 | 35.7 | 1.4637 | 30.6 | 1.4762 | 25.5 | 1.4891 | 20.4 |
| 1.4521 | 35.6 | 1.4639 | 30.5 | 1.4764 | 25.4 | 1.4893 | 20.3 |
| 1.4523 | 35.5 | 1.4642 | 30.4 | 1.4767 | 25.3 | 1.4896 | 20.2 |
| 1.4526 | 35.4 | 1.4644 | 30.3 | 1.4769 | 25.2 | 1.4898 | 20.1 |
| 1.4528 | 35.3 | 1.4646 | 30.2 | 1.4772 | 25.1 | **1.4901** | 20 |
| 1.4530 | 35.2 | 1.4649 | 30.1 | 1.4774 | 25 | 1.4904 | 19.9 |
| 1.4533 | 35.1 | 1.4651 | 30 | 1.4777 | 24.9 | 1.4906 | 19.8 |
| 1.4535 | 35 | 1.4653 | 29.9 | 1.4779 | 24.8 | 1.4909 | 19.7 |

TABLE 5. (*Concluded.*)

| Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. | Refractive index at 20° C. | Water. |
|---|---|---|---|---|---|---|---|
| | Per cent. | | Per cent. | | Per cent. | | Per cent. |
| 1.4912 | 19.6 | 1.4943 | 18.4 | 1.4975 | 17.2 | 1.5007 | 16 |
| 1.4914 | 19.5 | 1.4946 | 18.3 | 1.4978 | 17.1 | 1.5009 | 15.9 |
| 1.4917 | 19.4 | 1.4949 | 18.2 | 1.4980 | 17 | 1.5012 | 15.8 |
| 1.4919 | 19.3 | 1.4951 | 18.1 | 1.4983 | 16.9 | 1.5015 | 15.7 |
| 1.4922 | 19.2 | 1.4954 | 18 | 1.4985 | 16.8 | 1.5017 | 15.6 |
| 1.4925 | 19.1 | 1.4956 | 17.9 | 1.4988 | 16.7 | 1.5020 | 15.5 |
| 1.4927 | 19 | 1.4959 | 17.8 | 1.4991 | 16.6 | 1.5022 | 15.4 |
| 1.4930 | 18.9 | 1.4962 | 17.7 | 1.4993 | 16.5 | 1.5025 | 15.3 |
| 1.4933 | 18.8 | 1.4964 | 17.6 | 1.4996 | 16.4 | 1.5028 | 15.2 |
| 1.4935 | 18.7 | 1.4967 | 17.5 | 1.4999 | 16.3 | 1.5030 | 15.1 |
| 1.4938 | 18.6 | 1.4970 | 17.4 | 1.5001 | 16.2 | 1.5033 | 15 |
| 1.4941 | 18.5 | 1.4972 | 17.3 | 1.5004 | 16.1 | | |

TABLE* 6.

STANEK'S CORRECTION TABLE.

*For Determining Water in Sugar Solutions by Means of the Abbe Refractometer when Readings are Made at Other Temperatures than 20° C.*

| Water, per cent. | 95 | 90 | 85 | 80 | 70 | 60 | 50 | 40 | 30 | 25 | Water, per cent. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature °C. | To be added to the per cent of water. | | | | | | | | | | Temperature °C. |
| 15 | 0.25 | 0.27 | 0.31 | 0.31 | 0.34 | 0.35 | 0.36 | 0.37 | 0.36 | 0.36 | 15 |
| 16 | 0.21 | 0.23 | 0.26 | 0.27 | 0.29 | 0.31 | 0.31 | 0.32 | 0.31 | 0.29 | 16 |
| 17 | 0.16 | 0.18 | 0.20 | 0.20 | 0.22 | 0.23 | 0.23 | 0.23 | 0.20 | 0.17 | 17 |
| 18 | 0.11 | 0.12 | 0.14 | 0.14 | 0.15 | 0.16 | 0.16 | 0.15 | 0.12 | 0.09 | 18 |
| 19 | 0.06 | 0.07 | 0.08 | 0.08 | 0.08 | 0.09 | 0.09 | 0.08 | 0.07 | 0.05 | 19 |
| Temperature °C. | To be subtracted from the per cent of water. | | | | | | | | | | Temperature °C. |
| 21 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 21 |
| 22 | 0.12 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.15 | 0.14 | 0.14 | 0.14 | 22 |
| 23 | 0.18 | 0.20 | 0.20 | 0.21 | 0.21 | 0.21 | 0.23 | 0.21 | 0.22 | 0.22 | 23 |
| 24 | 0.24 | 0.26 | 0.26 | 0.27 | 0.28 | 0.28 | 0.30 | 0.28 | 0.29 | 0.29 | 24 |
| 25 | 0.30 | 0.32 | 0.32 | 0.34 | 0.36 | 0.36 | 0.38 | 0.36 | 0.36 | 0.37 | 25 |
| 26 | 0.36 | 0.39 | 0.39 | 0.41 | 0.43 | 0.43 | 0.46 | 0.44 | 0.43 | 0.44 | 26 |
| 27 | 0.43 | 0.46 | 0.46 | 0.48 | 0.50 | 0.51 | 0.55 | 0.62 | 0.50 | 0.51 | 27 |
| 28 | 0.50 | 0.53 | 0.53 | 0.55 | 0.58 | 0.59 | 0.63 | 0.70 | 0.57 | 0.59 | 28 |
| 29 | 0.57 | 0.60 | 0.61 | 0.62 | 0.66 | 0.67 | 0.71 | 0.78 | 0.65 | 0.67 | 29 |
| 30 | 0.64 | 0.67 | 0.70 | 0.71 | 0.74 | 0.75 | 0.80 | 0.86 | 0.73 | 0.75 | 30 |
| Water, per cent. | 95 | 90 | 85 | 80 | 70 | 60 | 50 | 40 | 30 | 25 | Water, per cent. |

* *See* "Handbook," page 64.

TABLE * 7.

GEERLIGS'S TABLE FOR DETERMINING DRY SUBSTANCE IN SUGAR-HOUSE PRODUCTS.

*By the Abbe Refractometer, at* 28° C.

| Refractive Index. | Per cent dry substance. | Decimals. | | Refractive Index. | Per cent dry substance. | Decimals. | |
|---|---|---|---|---|---|---|---|
| 1.3335 | 1 | 0.0001=0.05 | 0.0010=0.75 | 1.4104 | 46 | 0.0005=0.25 | 0.0016=0.8 |
| 1.3349 | 2 | 0.0002=0.1 | 0.0011=0.8 | 1.4124 | 47 | 0.0006=0.3 | 0.0017=0.85 |
| 1.3364 | 3 | 0.0003=0.2 | 0.0012=0.8 | 1.4145 | 48 | 0.0007=0.35 | 0.0018=0.9 |
| 1.3379 | 4 | 0.0004=0.25 | 0.0013=0.85 | 1.4166 | 49 | 0.0008=0.4 | 0.0019=0.95 |
| 1.3394 | 5 | 0.0005=0.3 | 0.0014=0.9 | 1.4186 | 50 | 0.0009=0.45 | 0.0020=1.0 |
| 1.3409 | 6 | 0.0006=0.4 | 0.0015=1.0 | 1.4207 | 51 | 0.0010=0.5 | 0.0021=1.0 |
| 1.3424 | 7 | 0.0007=0.5 | | 1.4228 | 52 | 0 0011=0.55 | |
| 1.3439 | 8 | 0.0008=0.6 | | 1.4249 | 53 | | |
| 1.3454 | 9 | 0.0009=0.7 | | 1.4270 | 54 | | |
| 1.3469 | 10 | | | | | | |
| | | | | 1.4292 | 55 | 0.0001=0.05 | 0.0013=0.55 |
| 1.3484 | 11 | 0.0001=0.05 | | 1.4314 | 56 | 0.0002=0.1 | 0.0014=0.6 |
| 1.3500 | 12 | 0.0002=0.1 | | 1.4337 | 57 | 0.0003=0.1 | 0.0015=0.65 |
| 1.3516 | 13 | 0.0003=0.2 | | 1.4359 | 58 | 0.0004=0.15 | 0.0016=0.7 |
| 1.3530 | 14 | 0.0004=0.25 | | 1.4382 | 59 | 0.0005=0.2 | 0.0017=0.75 |
| 1.3546 | 15 | 0.0005=0.3 | | 1.4405 | 60 | 0.0006=0.25 | 0.0018=0.8 |
| 1.3562 | 16 | 0.0006=0.4 | | 1.4428 | 61 | 0.0007=0.3 | 0.0019=0.85 |
| 1.3578 | 17 | 0.0007=0.45 | | 1.4451 | 62 | 0.0008=0.35 | 0.0020=0.9 |
| 1.3594 | 18 | 0.0008=0.5 | | 1.4474 | 63 | 0.0009=0.4 | 0.0021=0.9 |
| 1.3611 | 19 | 0.0009=0.6 | | 1.4497 | 64 | 0.0010=0.45 | 0.0022=0.95 |
| 1.3627 | 20 | 0.0010=0.65 | | 1.4520 | 65 | 0.0011=0.5 | 0.0023=1.0 |
| 1.3644 | 21 | 0.0011=0.7 | | 1.4543 | 66 | 0.0012=0.5 | 0.0024=1.0 |
| 1.3661 | 22 | 0.0012=0.75 | | 1.4567 | 67 | | |
| 1.3678 | 23 | 0.0013=0.8 | | 1.4591 | 68 | | |
| 1.3695 | 24 | 0.0014=0.85 | | 1.4615 | 69 | | |
| 1.3712 | 25 | 0.0015=0.9 | | 1.4639 | 70 | | |
| 1.3729 | 26 | 0.0016=0.95 | | 1.4663 | 71 | | |
| | | | | 1.4687 | 72 | | |
| 1.3746 | 27 | 0.0001=0.05 | 0.0012=0.6 | | | | |
| 1.3764 | 28 | 0.0002=0.1 | 0.0013=0.65 | 1.4711 | 73 | 0.0001=0.0 | 0.0015=0.55 |
| 1.3782 | 29 | 0.0003=0.15 | 0.0014=0.7 | 1.4736 | 74 | 0.0002=0.05 | 0.0016=0.6 |
| 1.3800 | 30 | 0.0004=0.2 | 0.0015=0.75 | 1.4761 | 75 | 0.0003=0.1 | 0.0017=0.65 |
| 1.3818 | 31 | 0.0005=0.25 | 0.0016=0.8 | 1.4786 | 76 | 0.0004=0.15 | 0.0018=0.65 |
| 1.3836 | 32 | 0.0006=0.3 | 0.0017=0.85 | 1.4811 | 77 | 0.0005=0.2 | 0.0019=0.7 |
| 1.3854 | 33 | 0.0007=0.35 | 0.0018=0.9 | 1.4836 | 78 | 0.0006=0.2 | 0.0020=0.75 |
| 1.3872 | 34 | 0.0008=0.4 | 0.0019=0.95 | 1.4862 | 79 | 0.0007=0.25 | 0.0021=0.8 |
| 1.3890 | 35 | 0.0009=0.45 | 0.0020=1.0 | 1.4888 | 80 | 0.0008=0.3 | 0.0022=0.8 |
| 1.3909 | 36 | 0.0010=0.5 | 0.0021=1.0 | 1.4914 | 81 | 0.0009=0.35 | 0.0023=0.85 |
| 1.3928 | 37 | 0.0011=0.55 | | 1.4940 | 82 | 0.0010=0.35 | 0.0024=0.9 |
| 1.3947 | 38 | | | 1.4966 | 83 | 0.0011=0.4 | 0.0025=0.9 |
| 1.3966 | 39 | | | 1.4992 | 84 | 0.0012=0.45 | 0.0026=0.95 |
| 1.3984 | 40 | | | 1.5019 | 85 | 0.0013=0.5 | 0.0027=1.0 |
| 1.4003 | 41 | | | 1.5046 | 86 | 0.0014=0.5 | 0.0028=1.0 |
| | | | | 1.5073 | 87 | | |
| 1.4023 | 42 | 0.0001=0.05 | 0.0012=0.6 | 1.5100 | 88 | | |
| 1.4043 | 43 | 0.0002=0.1 | 0.0013=0.65 | 1.5127 | 89 | | |
| 1.4063 | 44 | 0.0003=0.15 | 0.0014=0.7 | 1.5155 | 90 | | |
| 1.4083 | 45 | 0.0004=0.2 | 0.0015=0.75 | | | | |

* *See* "Handbook" page 65.

TABLE 7. (*Concluded.*)

CORRECTIONS FOR TEMPERATURE.

| Temperature of the prisms in ° C. | Dry substance. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
| | Subtract. | | | | | | | | | | | | |
| 20 | 0.53 | 0.54 | 0.55 | 0.56 | 0.57 | 0.58 | 0.60 | 0.62 | 0.64 | 0.62 | 0.61 | 0.60 | 0.58 |
| 21 | 0.46 | 0.47 | 0.48 | 0.49 | 0.50 | 0.51 | 0.52 | 0.54 | 0.56 | 0.54 | 0.53 | 0.52 | 0.50 |
| 22 | 0.40 | 0.41 | 0.42 | 0.42 | 0.43 | 0.44 | 0.45 | 0.47 | 0.48 | 0.47 | 0.46 | 0.45 | 0.44 |
| 23 | 0.33 | 0.33 | 0.34 | 0.35 | 0.36 | 0.37 | 0.38 | 0.39 | 0.40 | 0.39 | 0.38 | 0.38 | 0.38 |
| 24 | 0.26 | 0.26 | 0.27 | 0.28 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 | 0.31 | 0.31 | 0.30 | 0.30 |
| 25 | 0.20 | 0.20 | 0.21 | 0.21 | 0.22 | 0.22 | 0.23 | 0.23 | 0.24 | 0.23 | 0.23 | 0.23 | 0.22 |
| 26 | 0.12 | 0.12 | 0.13 | 0.14 | 0.14 | 0.14 | 0.15 | 0.15 | 0.16 | 0.16 | 0.16 | 0.15 | 0.14 |
| 27 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.07 |
| | Add. | | | | | | | | | | | | |
| 29 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.07 |
| 30 | 0.12 | 0.12 | 0.13 | 0.14 | 0.14 | 0.14 | 0.15 | 0.15 | 0.16 | 0.16 | 0.16 | 0.15 | 0.14 |
| 31 | 0.20 | 0.20 | 0.21 | 0.21 | 0.22 | 0.22 | 0.23 | 0.23 | 0.24 | 0.23 | 0.23 | 0.23 | 0.22 |
| 32 | 0.26 | 0.26 | 0.27 | 0.28 | 0.28 | 0.29 | 0.30 | 0.31 | 0.32 | 0.31 | 0.31 | 0.30 | 0.30 |
| 33 | 0.33 | 0.33 | 0.34 | 0.35 | 0.36 | 0.37 | 0.38 | 0.39 | 0.40 | 0.39 | 0.38 | 0.38 | 0.38 |
| 34 | 0.40 | 0.41 | 0.42 | 0.42 | 0.43 | 0.44 | 0.45 | 0.47 | 0.48 | 0.47 | 0.46 | 0.45 | 0.44 |
| 35 | 0.46 | 0.47 | 0.48 | 0.49 | 0.50 | 0.51 | 0.52 | 0.54 | 0.56 | 0.54 | 0.53 | 0.52 | 0.50 |

## TABLE * 8.

### Hübener's Table for Determining Percentages by Weight of Sucrose in Sugar Solutions from Readings of the Zeiss Immersion Refractometer.

| Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **15.0** | 0.00 | **21.0** | **1.58** | **27.0** | **3.16** | **33.0** | **4.74** | **39.0** | **6.31** | **45.0** | **7.84** | **51.0** | **9.32** |
| .1 | 0.03 | .1 | .61 | .1 | .19 | .1 | .77 | .1 | .33 | .1 | .87 | .1 | .34 |
| .2 | 0.05 | .2 | .64 | .2 | .21 | .2 | .79 | .2 | .36 | .2 | .90 | .2 | .36 |
| .3 | 0.08 | .3 | .66 | .3 | .24 | .3 | .82 | .3 | .39 | .3 | .92 | .3 | .39 |
| .4 | 0.11 | .4 | .69 | .4 | .26 | .4 | .84 | .4 | .41 | .4 | .95 | .4 | .41 |
| .5 | 0.13 | .5 | .71 | .5 | .29 | .5 | .87 | .5 | .43 | .5 | .97 | .5 | .44 |
| .6 | 0.16 | .6 | .74 | .6 | .32 | .6 | .90 | .6 | .46 | .6 | **8.00** | .6 | .46 |
| .7 | 0.19 | .7 | .77 | .7 | .34 | .7 | .92 | .7 | .49 | .7 | .03 | .7 | .49 |
| .8 | 0.21 | .8 | .79 | .8 | .37 | .8 | .95 | .8 | .51 | .8 | .05 | .8 | .51 |
| .9 | 0.24 | .9 | .82 | .9 | .40 | .9 | .98 | .9 | .54 | .9 | .07 | .9 | .53 |
| **16.0** | 0.26 | **22.0** | .84 | **28.0** | .42 | **34.0** | **5.00** | **40.0** | .56 | **46.0** | .10 | **52.0** | .56 |
| .1 | 0.29 | .1 | .87 | .1 | .45 | .1 | .03 | .1 | .59 | .1 | .12 | .1 | .58 |
| .2 | 0.32 | .2 | .90 | .2 | .48 | .2 | .05 | .2 | .61 | .2 | .15 | .2 | .60 |
| .3 | 0.34 | .3 | .92 | .3 | .50 | .3 | .08 | .3 | .64 | .3 | .17 | .3 | .63 |
| .4 | 0.37 | .4 | .95 | .4 | .53 | .4 | .11 | .4 | .66 | .4 | .19 | .4 | .66 |
| .5 | 0.40 | .5 | .98 | .5 | .56 | .5 | .13 | .5 | .69 | .5 | .22 | .5 | .68 |
| .6 | 0.42 | .6 | **2.00** | .6 | .58 | .6 | .16 | .6 | .72 | .6 | .24 | .6 | .70 |
| .7 | 0.45 | .7 | .03 | .7 | .61 | .7 | .19 | .7 | .74 | .7 | .27 | .7 | .73 |
| .8 | 0.48 | .8 | .05 | .8 | .64 | .8 | .21 | .8 | .77 | .8 | .29 | .8 | .75 |
| .9 | 0.50 | .9 | .08 | .9 | 66 | .9 | .24 | .9 | .79 | .9 | .32 | .9 | .78 |
| **17.0** | 0.53 | **23.0** | .11 | **29.0** | .69 | **35.0** | .26 | **41.0** | .82 | **47.0** | .34 | **53.0** | .80 |
| .1 | 0.56 | .1 | .13 | .1 | .71 | .1 | .29 | .1 | .84 | .1 | .36 | .1 | .83 |
| .2 | 0.58 | .2 | .16 | .2 | .74 | .2 | .32 | .2 | .87 | .2 | .39 | .2 | .85 |
| .3 | 0.61 | .3 | .19 | .3 | .77 | .3 | .34 | .3 | .90 | .3 | .41 | .3 | .88 |
| .4 | 0.64 | .4 | .21 | .4 | .79 | .4 | .37 | .4 | .92 | .4 | .44 | .4 | .90 |
| .5 | 0.66 | .5 | .24 | .5 | .82 | .5 | .40 | .5 | .95 | .5 | .46 | .5 | .92 |
| .6 | 0.69 | .6 | .26 | .6 | .84 | .6 | .42 | .6 | .97 | .6 | .49 | .6 | .95 |
| .7 | 0.71 | .7 | .29 | .7 | .87 | .7 | .45 | .7 | **7.00** | .7 | .51 | .7 | .97 |
| .8 | 0.74 | .8 | .32 | .8 | .90 | .8 | .48 | .8 | .03 | .8 | .53 | .8 | **10.00** |
| .9 | 0.77 | .9 | .34 | .9 | .92 | .9 | .50 | .9 | .05 | .9 | .56 | .9 | .03 |
| **18.0** | 0.79 | **24.0** | .37 | **30.0** | .95 | **36.0** | .53 | **42.0** | .08 | **48.0** | .58 | **54.0** | .05 |
| .1 | 0.82 | .1 | .40 | .1 | .98 | .1 | .56 | .1 | .10 | .1 | .60 | .1 | .07 |
| .2 | 0.84 | .2 | .42 | .2 | **4.00** | .2 | .58 | .2 | .13 | .2 | .63 | .2 | .10 |
| .3 | 0.87 | .3 | .45 | .3 | .03 | .3 | .61 | .3 | .15 | .3 | .66 | .3 | .12 |
| .4 | 0.90 | .4 | .48 | .4 | .05 | .4 | .64 | .4 | .18 | .4 | .68 | .4 | .15 |
| .5 | 0.92 | .5 | .50 | .5 | .08 | .5 | .66 | .5 | .20 | .5 | .70 | .5 | .17 |
| .6 | 0.95 | .6 | .53 | .6 | .11 | .6 | .69 | .6 | .23 | .6 | .73 | .6 | .19 |
| .7 | 0.98 | .7 | .56 | .7 | .13 | .7 | .71 | .7 | .26 | .7 | .75 | .7 | .22 |
| .8 | **1.00** | .8 | .58 | .8 | .16 | .8 | .74 | .8 | .28 | .8 | .78 | .8 | .24 |
| .9 | .03 | .9 | .61 | .9 | .19 | .9 | .77 | .9 | .31 | .9 | .80 | .9 | .27 |
| **19.0** | .05 | **25.0** | .64 | **31.0** | .21 | **37.0** | .79 | **43.0** | .33 | **49.0** | .83 | **55.0** | .29 |
| .1 | .08 | .1 | .66 | .1 | .24 | .1 | .82 | .1 | .36 | .1 | .85 | .1 | .32 |
| .2 | .11 | .2 | .69 | .2 | .26 | .2 | .84 | .2 | .39 | .2 | .88 | .2 | .34 |
| .3 | .13 | .3 | .71 | .3 | .29 | .3 | .87 | .3 | .41 | .3 | .90 | .3 | .36 |
| .4 | .16 | .4 | .74 | .4 | .32 | .4 | .90 | .4 | .43 | .4 | .92 | .4 | .39 |
| .5 | .19 | .5 | .77 | .5 | .34 | .5 | .92 | .5 | .46 | .5 | .95 | .5 | .41 |
| .6 | .21 | .6 | .79 | .6 | .37 | .6 | .95 | .6 | .49 | .6 | .97 | .6 | .44 |
| .7 | .24 | .7 | .82 | .7 | .39 | .7 | .98 | .7 | .51 | .7 | **9.00** | .7 | .46 |
| .8 | .26 | .8 | .84 | .8 | .42 | .8 | **6.00** | .8 | .54 | .8 | .03 | .8 | .49 |
| .9 | .29 | .9 | .87 | .9 | .45 | .9 | .03 | .9 | .56 | .9 | .05 | .9 | .51 |
| **20.0** | .32 | **26.0** | .90 | **32.0** | .48 | **38.0** | .05 | **44.0** | .59 | **50.0** | .07 | **56.0** | .53 |
| .1 | .34 | .1 | .92 | .1 | .50 | .1 | .08 | .1 | .61 | .1 | .10 | .1 | .56 |
| .2 | .37 | .2 | .95 | .2 | .53 | .2 | .10 | .2 | .64 | .2 | .12 | .2 | .58 |
| .3 | .40 | .3 | .98 | .3 | .56 | .3 | .13 | .3 | .66 | .3 | .15 | .3 | .60 |
| .4 | .42 | .4 | **3.00** | .4 | .58 | .4 | .15 | .4 | .69 | .4 | .17 | .4 | .63 |
| .5 | .45 | .5 | .03 | .5 | .61 | .5 | .17 | .5 | .72 | .5 | .19 | .5 | .66 |
| .6 | .48 | .6 | .05 | .6 | .64 | .6 | .20 | .6 | .74 | .6 | .22 | .6 | .68 |
| .7 | .50 | .7 | .08 | .7 | .66 | .7 | .23 | .7 | .77 | .7 | .24 | .7 | .70 |
| .8 | .53 | .8 | .11 | .8 | .69 | .8 | .26 | .8 | .79 | .8 | .27 | .8 | .73 |
| .9 | .56 | .9 | .13 | .9 | .71 | .9 | .28 | .9 | .82 | .9 | .29 | .9 | .75 |

* *See* "Handbook," page 74.

TABLE 8. (*Continued.*)

| Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **57.0** | **10.78** | **63.0** | **12.23** | **69.0** | **13.61** | **75.0** | **14.98** | **81.0** | **16.31** | **87.0** | **17.66** | **93.0** | **18.95** |
| .1 | .80 | .1 | .25 | .1 | .63 | .1 | **15.00** | .1 | .33 | .1 | .68 | .1 | .97 |
| .2 | .83 | .2 | .28 | .2 | .66 | .2 | .03 | .2 | .35 | .2 | .71 | .2 | **19.00** |
| .3 | .85 | .3 | .30 | .3 | .68 | .3 | .05 | .3 | .38 | .3 | .73 | .3 | .02 |
| .4 | .88 | .4 | .32 | .4 | .70 | .4 | .07 | .4 | .40 | .4 | .75 | .4 | .04 |
| .5 | .90 | .5 | .35 | .5 | .73 | .5 | .09 | .5 | .42 | .5 | .77 | .5 | .06 |
| .6 | .92 | .6 | .37 | .6 | .75 | .6 | .11 | .6 | .44 | .6 | .79 | .6 | .08 |
| .7 | .95 | .7 | .39 | .7 | .77 | .7 | .13 | .7 | .47 | .7 | .82 | .7 | .10 |
| .8 | .97 | .8 | .42 | .8 | .79 | .8 | .16 | .8 | .49 | .8 | .84 | .8 | .13 |
| .9 | **11.00** | .9 | .44 | .9 | .82 | .9 | .18 | .9 | .51 | .9 | .86 | .9 | .15 |
| **58.0** | .03 | **64.0** | .46 | **70.0** | .84 | **76.0** | .20 | **82.0** | .54 | **88.0** | .89 | **94.0** | .17 |
| .1 | .05 | .1 | .49 | .1 | .87 | .1 | .22 | .1 | .56 | .1 | .91 | .1 | .19 |
| .2 | .07 | .2 | .51 | .2 | .89 | .2 | .24 | .2 | .59 | .2 | .93 | .2 | .21 |
| .3 | .10 | .3 | .53 | .3 | .92 | .3 | .26 | .3 | .61 | .3 | .95 | .3 | .23 |
| .4 | .12 | .4 | .56 | .4 | .94 | .4 | .28 | .4 | .63 | .4 | .98 | .4 | .25 |
| .5 | .15 | .5 | .58 | .5 | .96 | .5 | .30 | .5 | .65 | .5 | **18.00** | .5 | .27 |
| .6 | .17 | .6 | .60 | .6 | .98 | .6 | .32 | .6 | .68 | .6 | .02 | .6 | .29 |
| .7 | .19 | .7 | .63 | .7 | **14.00** | .7 | .34 | .7 | .70 | .7 | .04 | .7 | .31 |
| .8 | .22 | .8 | .65 | .8 | .03 | .8 | .36 | .8 | .72 | .8 | .06 | .8 | .34 |
| .9 | .24 | .9 | .67 | .9 | .05 | .9 | .38 | .9 | .74 | .9 | .08 | .9 | .36 |
| **59.0** | .27 | **65.0** | .69 | **71.0** | .07 | **77.0** | .40 | **83.0** | .76 | **89.0** | .10 | **95.0** | .38 |
| .1 | .29 | .1 | .72 | .1 | .09 | .1 | .42 | .1 | .79 | .1 | .13 | .1 | .40 |
| .2 | .32 | .2 | .74 | .2 | .11 | .2 | .44 | .2 | .81 | .2 | .15 | .2 | .42 |
| .3 | .34 | .3 | .76 | .3 | .14 | .3 | .47 | .3 | .83 | .3 | .17 | .3 | .44 |
| .4 | .36 | .4 | .79 | .4 | .16 | .4 | .49 | .4 | .85 | .4 | .19 | .4 | .47 |
| .5 | .39 | .5 | .81 | .5 | .18 | .5 | .51 | .5 | .88 | .5 | .21 | .5 | .49 |
| .6 | .41 | .6 | .83 | .6 | .20 | .6 | .54 | .6 | .90 | .6 | .23 | .6 | .51 |
| .7 | .44 | .7 | .86 | .7 | .23 | .7 | .56 | .7 | .92 | .7 | .25 | .7 | .53 |
| .8 | .46 | .8 | .88 | .8 | .25 | .8 | .59 | .8 | .95 | .8 | .27 | .8 | .55 |
| .9 | .49 | .9 | .90 | .9 | .27 | .9 | .61 | .9 | .97 | .9 | .29 | .9 | .57 |
| **60.0** | .51 | **66.0** | .93 | **72.0** | .29 | **78.0** | .63 | **84.0** | **17.00** | **90.0** | .31 | **96.0** | .59 |
| .1 | .53 | .1 | .95 | .1 | .32 | .1 | .65 | .1 | .02 | .1 | .34 | .1 | .61 |
| .2 | .56 | .2 | .97 | .2 | .34 | .2 | .68 | .2 | .04 | .2 | .36 | .2 | .63 |
| .3 | .58 | .3 | **13.00** | .3 | .36 | .3 | .70 | .3 | .07 | .3 | .38 | .3 | .66 |
| .4 | .60 | .4 | .03 | .4 | .38 | .4 | .72 | .4 | .09 | .4 | .40 | .4 | .68 |
| .5 | .63 | .5 | .05 | .5 | .40 | .5 | .74 | .5 | .11 | .5 | .42 | .5 | .70 |
| .6 | .66 | .6 | .07 | .6 | .43 | .6 | .76 | .6 | .13 | .6 | .44 | .6 | .72 |
| .7 | .68 | .7 | .09 | .7 | .45 | .7 | .79 | .7 | .15 | .7 | .47 | .7 | .74 |
| .8 | .70 | .8 | .11 | .8 | .48 | .8 | .81 | .8 | .18 | .8 | .49 | .8 | .76 |
| .9 | .73 | .9 | .14 | .9 | .50 | .9 | .83 | .9 | .20 | .9 | .51 | .9 | .78 |
| **61.0** | .75 | **67.0** | .16 | **73.0** | .52 | **79.0** | .85 | **85.0** | .22 | **91.0** | .53 | **97.0** | .80 |
| .1 | .78 | .1 | .18 | .1 | .54 | .1 | .88 | .1 | .24 | .1 | .55 | .1 | .82 |
| .2 | .80 | .2 | .20 | .2 | .57 | .2 | .90 | .2 | .27 | .2 | .57 | .2 | .85 |
| .3 | .83 | .3 | .23 | .3 | .59 | .3 | .92 | .3 | .29 | .3 | .59 | .3 | .87 |
| .4 | .85 | .4 | .25 | .4 | .61 | .4 | .95 | .4 | .31 | .4 | .61 | .4 | .89 |
| .5 | .88 | .5 | .27 | .5 | .63 | .5 | .97 | .5 | .33 | .5 | .63 | .5 | .91 |
| .6 | .90 | .6 | .29 | .6 | .66 | .6 | **16.00** | .6 | .35 | .6 | .66 | .6 | .93 |
| .7 | .92 | .7 | .32 | .7 | .68 | .7 | .03 | .7 | .38 | .7 | .68 | .7 | .95 |
| .8 | .95 | .8 | .34 | .8 | .70 | .8 | .05 | .8 | .40 | .8 | .70 | .8 | .97 |
| .9 | .97 | .9 | .36 | .9 | .73 | .9 | .07 | .9 | .42 | .9 | .72 | .9 | **20.00** |
| **62.0** | **12.00** | **68.0** | .38 | **74.0** | .75 | **80.0** | .09 | **86.0** | .44 | **92.0** | .74 | **98.0** | .02 |
| .1 | .03 | .1 | .40 | .1 | .77 | .1 | .11 | .1 | .47 | .1 | .76 | .1 | .04 |
| .2 | .05 | .2 | .43 | .2 | .79 | .2 | .13 | .2 | .49 | .2 | .78 | .2 | .06 |
| .3 | .07 | .3 | .45 | .3 | .82 | .3 | .16 | .3 | .51 | .3 | .80 | .3 | .08 |
| .4 | .09 | .4 | .48 | .4 | .84 | .4 | .18 | .4 | .53 | .4 | .82 | .4 | .10 |
| .5 | .12 | .5 | .50 | .5 | .87 | .5 | .20 | .5 | .55 | .5 | .85 | .5 | .13 |
| .6 | .14 | .6 | .52 | .6 | .89 | .6 | .22 | .6 | .58 | .6 | .87 | .6 | .15 |
| .7 | .16 | .7 | .54 | .7 | .92 | .7 | .24 | .7 | .60 | .7 | .89 | .7 | .17 |
| .8 | .18 | .8 | .57 | .8 | .94 | .8 | .27 | .8 | .62 | .8 | .91 | .8 | .19 |
| .9 | .21 | .9 | .59 | .9 | .96 | .9 | .29 | .9 | .64 | .9 | .93 | .9 | .21 |

TABLE 8. (*Concluded*).

| Scale reading of refractometer. | Per cent sucrose | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. | Scale reading of refractometer. | Per cent sucrose. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **99.0** | **20.23** | **100.0** | **20.44** | **101.0** | **20.66** | **102.0** | **20.87** | **103.0** | **21.08** | **104.0** | **21.29** | **105.0** | **21.51** |
| .1 | .25 | .1 | .47 | .1 | .68 | .1 | .89 | .1 | .10 | .1 | .31 | .1 | .53 |
| .2 | .27 | .2 | .49 | .2 | .70 | .2 | .91 | .2 | .13 | .2 | .34 | .2 | .55 |
| .3 | .29 | .3 | .51 | .3 | .72 | .3 | .93 | .3 | .15 | .3 | .36 | .3 | .57 |
| .4 | .31 | .4 | .53 | .4 | .74 | .4 | .95 | .4 | .17 | .4 | .38 | .4 | .59 |
| .5 | .34 | .5 | .55 | .5 | .76 | .5 | .97 | .5 | .19 | .5 | .40 | .5 | .61 |
| .6 | .36 | .6 | .57 | .6 | .78 | .6 | **21.00** | .6 | .21 | .6 | .42 | .6 | .63 |
| .7 | .38 | .7 | .59 | .7 | .80 | .7 | .02 | .7 | .23 | .7 | .44 | .7 | .66 |
| .8 | .40 | .8 | .61 | .8 | .82 | .8 | .04 | .8 | .25 | .8 | .47 | .8 | .68 |
| .9 | .42 | .9 | .63 | .9 | .85 | .9 | .06 | .9 | .27 | .9 | .49 | .9 | .70 |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | **106.0** | **21.71** |

TABLE* 9.

KRUIS'S TABLE FOR DETERMINING GLUCOSE BY REISCHAUER'S METHOD.

| Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. |
|---|---|---|---|---|---|---|---|
| c.c. | mgs. | c.c. | mgs. | c.c | mgs. | c.c. | mgs. |
| **1.00** | 5.57 | 1.53 | 8.20 | 2.06 | 10.64 | 2.59 | 13.06 |
| 1.01 | 5.64 | 1.54 | 8.24 | 2.07 | 10.68 | **2.60** | 13.11 |
| 1.02 | 5.81 | 1.55 | 8.29 | 2.08 | 10.73 | 2.61 | 13.16 |
| 1.03 | 5.85 | 1.56 | 8.34 | 2.09 | 10.77 | 2.62 | 13.20 |
| 1.04 | 5.90 | 1.57 | 8.38 | **2.10** | 10.82 | 2.63 | 13.25 |
| 1.05 | 5.94 | 1.58 | 8.43 | 2.11 | 10.87 | 2.64 | 13.29 |
| 1.06 | 5.99 | 1.59 | 8.48 | 2.12 | 10.91 | 2.65 | 13.34 |
| 1.07 | 6.04 | **1.60** | 8.52 | 2.13 | 10.96 | 2.66 | 13.39 |
| 1.08 | 6.08 | 1.61 | 8.57 | 2.14 | 11.00 | 2.67 | 13.43 |
| 1.09 | 6.13 | 1.62 | 8.62 | 2.15 | 11.04 | 2.68 | 13.48 |
| **1.10** | 6.18 | 1.63 | 8.66 | 2.16 | 11.09 | 2.69 | 13.52 |
| 1.11 | 6.22 | 1.64 | 8.71 | 2.17 | 11.14 | **2.70** | 13.57 |
| 1.12 | 6.27 | 1.65 | 8.76 | 2.18 | 11.18 | 2.71 | 13.62 |
| 1.13 | 6.32 | 1.66 | 8.80 | 2.19 | 11.23 | 2.72 | 13.66 |
| 1.14 | 6.36 | 1.67 | 8.85 | **2.20** | 11.28 | 2.73 | 13.71 |
| 1.15 | 6.41 | 1.68 | 8.89 | 2.21 | 11.32 | 2.74 | 13.76 |
| 1.16 | 6.46 | 1.69 | 8.94 | 2.22 | 11.37 | 2.75 | 13.80 |
| 1.17 | 6.51 | **1.70** | 8.99 | 2.23 | 11.41 | 2.76 | 13.85 |
| 1.18 | 6.55 | 1.71 | 9.03 | 2.24 | 11.46 | 2.77 | 13.89 |
| 1.19 | 6.60 | 1.72 | 9.08 | 2.25 | 11.50 | 2.78 | 13.94 |
| **1.20** | 6.65 | 1.73 | 9.13 | 2.26 | 11.55 | 2.79 | 13.99 |
| 1.21 | 6.69 | 1.74 | 9.17 | 2.27 | 11.60 | **2.80** | 14.03 |
| 1.22 | 6.74 | 1.75 | 9.22 | 2.28 | 11.64 | 2.81 | 14.08 |
| 1.23 | 6.79 | 1.76 | 9.26 | 2.29 | 11.69 | 2.82 | 14.12 |
| 1.24 | 6.84 | 1.77 | 9.31 | **2.30** | 11.73 | 2.83 | 14.17 |
| 1.25 | 6.88 | 1.78 | 9.36 | 2.31 | 11.78 | 2.84 | 14.22 |
| 1.26 | 6.93 | 1.79 | 9.40 | 2.32 | 11.82 | 2.85 | 14.26 |
| 1.27 | 6.98 | **1.80** | 9.45 | 2.33 | 11.87 | 2.86 | 14.31 |
| 1.28 | 7.02 | 1.81 | 9.49 | 2.34 | 11.92 | 2.87 | 14.35 |
| 1.29 | 7.07 | 1.82 | 9.54 | 2.35 | 12.96 | 2.88 | 14.40 |
| **1.30** | 7.12 | 1.83 | 9.59 | 2.36 | 12.00 | 2.89 | 14.45 |
| 1.31 | 7.17 | 1.84 | 9.63 | 2.37 | 12.05 | **2.90** | 14.49 |
| 1.32 | 7.21 | 1.85 | 9.68 | 2.38 | 12.10 | 2.91 | 14.54 |
| 1.33 | 7.26 | 1.86 | 9.72 | 2.39 | 12.14 | 2.92 | 14.58 |
| 1.34 | 7.31 | 1.87 | 9.77 | **2.40** | 12.19 | 2.93 | 14.63 |
| 1.35 | 7.35 | 1.88 | 9.81 | 2.41 | 12.24 | 2.94 | 14.68 |
| 1.36 | 7.40 | 1.89 | 9.86 | 2.42 | 12.28 | 2.95 | 14.72 |
| 1.37 | 7.45 | **1.90** | 9.91 | 2.43 | 12.33 | 2.96 | 14.77 |
| 1.38 | 7.49 | 1.91 | 9.95 | 2.44 | 12.37 | 2.97 | 14.81 |
| 1.39 | 7.54 | 1.92 | 10.00 | 2.45 | 12.42 | 2.98 | 14.86 |
| **1.40** | 7.59 | 1.93 | 10.04 | 2.46 | 12.47 | 2.99 | 14.91 |
| 1.41 | 7.64 | 1.94 | 10.09 | 2.47 | 12.51 | **3.00** | 14.95 |
| 1.42 | 7.68 | 1.95 | 10.13 | 2.48 | 12.56 | 3.01 | 15.00 |
| 1.43 | 7.73 | 1.96 | 10.18 | 2.49 | 12.60 | 3.02 | 15.04 |
| 1.44 | 7.77 | 1.97 | 10.23 | **2.50** | 12.65 | 3.03 | 15.09 |
| 1.45 | 7.82 | 1.98 | 10.27 | 2.51 | 12.69 | 3.04 | 15.14 |
| 1.46 | 7.87 | 1.99 | 10.32 | 2.52 | 12.74 | 3.05 | 15.18 |
| 1.47 | 7.92 | **2.00** | 10.36 | 2.53 | 12.79 | 3.06 | 15.23 |
| 1.48 | 7.96 | 2.01 | 10.41 | 2.54 | 12.83 | 3.07 | 15.27 |
| 1.49 | 8.01 | 2.02 | 10.45 | 2.55 | 12.88 | 3.08 | 15.32 |
| **1.50** | 8.06 | 2.03 | 10.50 | 2.56 | 12.92 | 3.09 | 15.37 |
| 1.51 | 8.10 | 2.04 | 10.55 | 2.57 | 12.97 | **3.10** | 15.41 |
| 1.52 | 8.15 | 2.05 | 10.59 | 2.58 | 13.02 | 3.11 | 15.46 |

* *See* "Handbook," page 398.

TABLE 9. (*Continued.*)

| Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. |
|---|---|---|---|---|---|---|---|
| c.c. | mgs. | c.c. | mgs. | c.c. | mgs. | c.c. | mgs. |
| 3.12 | 15.50 | 3.65 | 17.95 | 4.18 | 20.41 | 4.71 | 22.90 |
| 3.13 | 15.55 | 3.66 | 17.99 | 4.19 | 20.46 | 4.72 | 22.94 |
| 3.14 | 15.60 | 3.67 | 18.04 | **4.20** | 20.51 | 4.73 | 22.99 |
| 3.15 | 15.64 | 3.68 | 18.09 | 4.21 | 20.55 | 4.74 | 23.04 |
| 3.16 | 15.69 | 3.69 | 18.13 | 4.22 | 20.60 | 4.75 | 23.09 |
| 3.17 | 15.73 | **3.70** | 18.18 | 4.23 | 20.65 | 4.76 | 23.13 |
| 3.18 | 15.78 | 3.71 | 18.23 | 4.24 | 20.69 | 4.77 | 23.18 |
| 3.19 | 15.83 | 3.72 | 18.27 | 4.25 | 20.74 | 4.78 | 23.23 |
| **3.20** | 15.87 | 3.73 | 18.32 | 4.26 | 20.79 | 4.79 | 23.28 |
| 3.21 | 15.92 | 3.74 | 18.37 | 4.27 | 20.83 | **4.80** | 23.32 |
| 3.22 | 15.96 | 3.75 | 18.41 | 4.28 | 20.88 | 4.81 | 23.37 |
| 3.23 | 16.01 | 3.76 | 18.46 | 4.29 | 20.93 | 4.82 | 23.42 |
| 3.24 | 16.06 | 3.77 | 18.50 | **4.30** | 20.98 | 4.83 | 23.46 |
| 3.25 | 16.10 | 3.78 | 18.55 | 4.31 | 21.02 | 4.84 | 23.51 |
| 3.26 | 16.15 | 3.79 | 18.60 | 4.32 | 21.07 | 4.85 | 23.56 |
| 3.27 | 16.19 | **3.80** | 18.64 | 4.33 | 21.12 | 4.86 | 23.60 |
| 3.28 | 16.24 | 3.81 | 18.69 | 4.34 | 21.16 | 4.87 | 23.65 |
| 3.29 | 16.29 | 3.82 | 18.73 | 4.35 | 21.21 | 4.88 | 23.70 |
| **3.30** | 16.33 | 3.83 | 18.78 | 4.36 | 21.26 | 4.89 | 23.74 |
| 3.31 | 16.38 | 3.84 | 18.83 | 4.37 | 21.30 | **4.90** | 23.79 |
| 3.32 | 16.43 | 3.85 | 18.88 | 4.38 | 21.35 | 4.91 | 23.84 |
| 3.33 | 16.47 | 3.86 | 18.92 | 4.39 | 21.40 | 4.92 | 23.89 |
| 3.34 | 16.52 | 3.87 | 18.97 | **4.40** | 21.44 | 4.93 | 23.93 |
| 3.35 | 16.56 | 3.88 | 19.02 | 4.41 | 21.49 | 4.94 | 23.98 |
| 3.36 | 16.61 | 3.89 | 19.06 | 4.42 | 21.54 | 4.95 | 24.03 |
| 3.37 | 16.66 | **3.90** | 19.11 | 4.43 | 21.58 | 4.96 | 24.07 |
| 3.38 | 16.70 | 3.91 | 19.15 | 4.44 | 21.63 | 4.97 | 24.12 |
| 3.39 | 16.75 | 3.92 | 19.20 | 4.45 | 21.68 | 4.98 | 24.17 |
| **3.40** | 16.79 | 3.93 | 19.25 | 4.46 | 21.73 | 4.99 | 24.22 |
| 3.41 | 16.84 | 3.94 | 19.29 | 4.47 | 21.77 | **5.00** | 24.26 |
| 3.42 | 16.89 | 3.95 | 19.34 | 4.48 | 21.82 | 5.01 | 24.31 |
| 3.43 | 16.93 | 3.96 | 19.39 | 4.49 | 21.87 | 5.02 | 24.36 |
| 3.44 | 16.98 | 3.97 | 19.43 | **4.50** | 21.91 | 5.03 | 24.40 |
| 3.45 | 17.02 | 3.98 | 19.48 | 4.51 | 21.96 | 5.04 | 24.45 |
| 3.46 | 17.07 | 3.99 | 19.53 | 4.52 | 22.01 | 5.05 | 24.50 |
| 3.47 | 17.12 | **4.00** | 19.57 | 4.53 | 22.05 | 5.06 | 24.55 |
| 3.48 | 17.16 | 4.01 | 19.62 | 4.54 | 22.10 | 5.07 | 24.59 |
| 3.49 | 17.21 | 4.02 | 19.67 | 4.55 | 22.14 | 5.08 | 24.64 |
| **3.50** | 17.26 | 4.03 | 19.71 | 4.56 | 22.19 | 5.09 | 24.69 |
| 3.51 | 17.30 | 4.04 | 19.76 | 4.57 | 22.24 | **5.10** | 24.73 |
| 3.52 | 17.35 | 4.05 | 19.80 | 4.58 | 22.29 | 5.11 | 24.78 |
| 3.53 | 17.39 | 4.06 | 19.85 | 4.59 | 22.34 | 5.12 | 24.83 |
| 3.54 | 17.44 | 4.07 | 19.90 | **4.60** | 22.38 | 5.13 | 24.88 |
| 3.55 | 17.49 | 4.08 | 19.95 | 4.61 | 22.43 | 5.14 | 24.92 |
| 3.56 | 17.53 | 4.09 | 19.99 | 4.62 | 22.48 | 5.15 | 24.97 |
| 3.57 | 17.58 | **4.10** | 20.04 | 4.63 | 22.52 | 5.16 | 25.02 |
| 3.58 | 17.62 | 4 11 | 20.09 | 4.64 | 22.57 | 5.17 | 25.06 |
| 3.59 | 17.67 | 4.12 | 20.13 | 4.65 | 22.62 | 5.18 | 25.11 |
| **3.60** | 17.72 | 4.13 | 20.18 | 4.66 | 22.66 | 5.19 | 25.16 |
| 3.61 | 17.76 | 4.14 | 20.23 | 4.67 | 22.71 | **5.20** | 25.20 |
| 3.62 | 17.81 | 4.15 | 20.27 | 4.68 | 22.76 | 5.21 | 25.25 |
| 3.63 | 17.86 | 4.16 | 20.32 | 4.69 | 22.80 | 5.22 | 25.30 |
| 3.64 | 17.90 | 4.17 | 20.37 | **4.70** | 22.85 | 5.23 | 25.34 |

TABLE 9. (*Concluded.*)

| Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. | Fehling's solution. | Glucose. |
|---|---|---|---|---|---|---|---|
| c.c. | mgs. | c.c. | mgs. | c.c. | mgs. | c.c. | mgs. |
| 5.24 | 25.39 | 5.44 | 26.34 | 5.64 | 27.28 | 5.84 | 28.22 |
| 5.25 | 25.44 | 5.45 | 26.38 | 5.65 | 27.32 | 5.85 | 28.26 |
| 5.26 | 25.49 | 5.46 | 26.43 | 5.66 | 27.37 | 5.86 | 28.31 |
| 5.27 | 25.53 | 5.47 | 26.48 | 5.67 | 27.42 | 5.87 | 28.36 |
| 5.28 | 25.58 | 5.48 | 26.52 | 5.68 | 27.47 | 5.88 | 28.41 |
| 5.29 | 25.63 | 5.49 | 26.57 | 5.69 | 27.51 | 5.89 | 28.46 |
| **5.30** | 25.68 | **5.50** | 26.62 | **5.70** | 27.56 | **5.90** | 28.50 |
| 5.31 | 25.72 | 5.51 | 26.66 | 5.71 | 27.61 | 5.91 | 28.55 |
| 5.32 | 25.77 | 5.52 | 26.72 | 5.72 | 27.65 | 5.92 | 28.60 |
| 5.33 | 25.82 | 5.53 | 26.76 | 5.73 | 27.70 | 5.93 | 28.64 |
| 5.34 | 25.86 | 5.54 | 26.81 | 5.74 | 27.75 | 5.94 | 28.69 |
| 5.35 | 25.91 | 5.55 | 26.85 | 5.75 | 27.80 | 5.95 | 28.74 |
| 5.36 | 25.96 | 5.56 | 26.90 | 5.76 | 27.84 | 5.96 | 28.79 |
| 5.37 | 26.00 | 5.57 | 26.95 | 5.77 | 27.89 | 5.97 | 28.83 |
| 5.38 | 26.05 | 5.58 | 26.99 | 5.78 | 27.90 | 5.98 | 28.88 |
| 5.39 | 26.10 | 5.59 | 27.04 | 5.79 | 27.98 | 5.99 | 28.93 |
| **5.40** | 26.15 | **5.60** | 27.09 | **5.80** | 28.03 | **6.00** | 28.97 |
| 5.41 | 26.19 | 5.61 | 27.14 | 5.81 | 28.08 | | |
| 5.42 | 26.24 | 5.62 | 27.19 | 5.82 | 28.13 | | |
| 5.43 | 26.29 | 5.63 | 27.23 | 5.83 | 28.17 | | |

## TABLE* 10.

### Allihn's Table for Determining Glucose.

| Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu.) | Cuprous oxide. ($Cu_2O$). | Glucose. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 11 | 12.4 | 6.6 | 51 | 57.4 | 26.4 | 91 | 102.4 | 46.4 | 131 | 147.5 | 66.7 |
| 12 | 13.5 | 7.1 | 52 | 58.5 | 26.9 | 92 | 103.6 | 46.9 | 132 | 148.6 | 67.2 |
| 13 | 14.6 | 7.6 | 53 | 59.7 | 27.4 | 93 | 104.7 | 47.4 | 133 | 149.7 | 67.7 |
| 14 | 15.8 | 8.1 | 54 | 60.8 | 27.9 | 94 | 105.8 | 47.9 | 134 | 150.9 | 68.2 |
| 15 | 16.9 | 8.6 | 55 | 61.9 | 28.4 | 95 | 107.0 | 48.4 | 135 | 152.0 | 68.8 |
| 16 | 18.0 | 9.0 | 56 | 63.0 | 28.8 | 96 | 108.1 | 48.9 | 136 | 153.1 | 69.3 |
| 17 | 19.1 | 9.5 | 57 | 64.2 | 29.3 | 97 | 109.2 | 49.4 | 137 | 154.2 | 69.8 |
| 18 | 20.3 | 10.0 | 58 | 65.3 | 29.8 | 98 | 110.3 | 49.9 | 138 | 155.4 | 70.3 |
| 19 | 21.4 | 10.5 | 59 | 66.4 | 30.3 | 99 | 111.5 | 50.4 | 139 | 156.5 | 70.8 |
| 20 | 22.5 | 11.0 | 60 | 67.6 | 30.8 | 100 | 112.6 | 50.9 | 140 | 157.6 | 71.3 |
| 21 | 23.6 | 11.5 | 61 | 68.7 | 31.3 | 101 | 113.7 | 51.4 | 141 | 158.7 | 71.8 |
| 22 | 24.8 | 12.0 | 62 | 69.8 | 31.8 | 102 | 114.8 | 51.9 | 142 | 159.9 | 72.3 |
| 23 | 25.9 | 12.5 | 63 | 70.9 | 32.3 | 103 | 116.0 | 52.4 | 143 | 161.0 | 72.9 |
| 24 | 27.0 | 13.0 | 64 | 72.1 | 32.8 | 104 | 117.1 | 52.9 | 144 | 162.1 | 73.4 |
| 25 | 28.1 | 13.5 | 65 | 73.2 | 33.3 | 105 | 118.2 | 53.5 | 145 | 163.2 | 73.9 |
| 26 | 29.3 | 14.0 | 66 | 74.3 | 33.3 | 106 | 119.3 | 54.0 | 146 | 164.4 | 74.4 |
| 27 | 30.4 | 14.5 | 67 | 75.4 | 34.3 | 107 | 120.5 | 54.5 | 147 | 165.5 | 74.9 |
| 28 | 31.5 | 15.0 | 68 | 76.6 | 34.8 | 108 | 121.6 | 55.0 | 148 | 166.6 | 75.5 |
| 29 | 32.7 | 15.5 | 69 | 77.7 | 35.3 | 109 | 122.7 | 55.5 | 149 | 167.7 | 76.0 |
| 30 | 33.8 | 16.0 | 70 | 78.8 | 35.8 | 110 | 123.8 | 56.0 | 150 | 168.9 | 76.5 |
| 31 | 34.9 | 16.5 | 71 | 79.9 | 36.3 | 111 | 125.0 | 56.5 | 151 | 170.0 | 77.0 |
| 32 | 36.0 | 17.0 | 72 | 81.1 | 36.8 | 112 | 126.1 | 57.0 | 152 | 171.1 | 77.5 |
| 33 | 37.2 | 17.5 | 73 | 82.2 | 37.3 | 113 | 127.2 | 57.5 | 153 | 172.3 | 78.1 |
| 34 | 38.3 | 18.0 | 74 | 83.3 | 37.8 | 114 | 128.3 | 58.0 | 154 | 173.4 | 78.6 |
| 35 | 39.4 | 18.5 | 75 | 84.4 | 38.3 | 115 | 129.6 | 58.6 | 155 | 174.5 | 79.1 |
| 36 | 40.5 | 18.9 | 76 | 85.6 | 38.8 | 116 | 130.6 | 59.1 | 156 | 175.6 | 79.6 |
| 37 | 41.7 | 19.4 | 77 | 86.7 | 39.3 | 117 | 131.7 | 59.6 | 157 | 176.8 | 80.1 |
| 38 | 42.8 | 19.9 | 78 | 87.8 | 39.8 | 118 | 132.8 | 60.1 | 158 | 177.9 | 80.7 |
| 39 | 43.9 | 20.4 | 79 | 88.9 | 40.3 | 119 | 134.0 | 60.6 | 159 | 179.0 | 81.2 |
| 40 | 45.0 | 20.9 | 80 | 90.1 | 40.8 | 120 | 135.1 | 61.1 | 160 | 180.1 | 81.7 |
| 41 | 46.2 | 21.4 | 81 | 91.2 | 41.3 | 121 | 136.2 | 61.6 | 161 | 181.3 | 82.2 |
| 42 | 47.3 | 21.9 | 82 | 92.3 | 41.8 | 122 | 137.4 | 62.1 | 162 | 182.4 | 82.7 |
| 43 | 48.4 | 22.4 | 83 | 93.4 | 42.3 | 123 | 138.5 | 62.6 | 163 | 183.5 | 83.3 |
| 44 | 49.5 | 22.9 | 84 | 94.6 | 42.8 | 124 | 139.6 | 63.1 | 164 | 184.6 | 83.8 |
| 45 | 50.7 | 23.4 | 85 | 95.7 | 43.4 | 125 | 140.7 | 63.7 | 165 | 185.8 | 84.3 |
| 46 | 51.8 | 23.9 | 86 | 96.8 | 43.9 | 126 | 141.9 | 64.2 | 166 | 186.9 | 84.8 |
| 47 | 52.9 | 24.4 | 87 | 97.9 | 44.4 | 127 | 143.0 | 64.7 | 167 | 188.0 | 85.3 |
| 48 | 54.0 | 24.9 | 88 | 99.1 | 44.9 | 128 | 144.1 | 65.2 | 168 | 189.1 | 85.9 |
| 49 | 55.2 | 25.4 | 89 | 100.2 | 45.4 | 129 | 145.2 | 65.7 | 169 | 190.3 | 86.4 |
| 50 | 56.3 | 25.9 | 90 | 101.3 | 45.9 | 130 | 146.4 | 66.2 | 170 | 191.4 | 86.9 |

* *See* "Handbook," page 403.

TABLE 10. (*Continued.*)

| Copper (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 171 | 192.5 | 87.4 | 216 | 243.2 | 111.1 | 261 | 293.8 | 135.1 | 306 | 344.5 | 159.8 |
| 172 | 193.6 | 87.9 | 217 | 244.3 | 111.6 | 262 | 295.0 | 135.7 | 307 | 345.6 | 160.4 |
| 173 | 194.8 | 88.5 | 218 | 245.4 | 112.1 | 263 | 296.1 | 136.2 | 308 | 346.8 | 160.9 |
| 174 | 195.9 | 89.0 | 219 | 246.6 | 112.7 | 264 | 297.2 | 136.8 | 309 | 347.9 | 161.5 |
| 175 | 197.0 | 89.5 | 220 | 247.7 | 113.2 | 265 | 298.3 | 137.3 | 310 | 349.0 | 162.0 |
| 176 | 198.1 | 90.0 | 221 | 248.7 | 113.7 | 266 | 299.5 | 137.8 | 311 | 350.1 | 162.6 |
| 177 | 199.3 | 90.5 | 222 | 249.9 | 114.3 | 267 | 300.6 | 138.4 | 312 | 351.3 | 163.1 |
| 178 | 200.4 | 91.1 | 223 | 251.0 | 114.8 | 268 | 301.7 | 138.9 | 313 | 352.4 | 163.7 |
| 179 | 201.5 | 91.6 | 224 | 252.4 | 115.3 | 269 | 302.8 | 139.5 | 314 | 353.5 | 164.2 |
| 180 | 202.6 | 92.1 | 225 | 253.3 | 115.9 | 270 | 304.0 | 140.0 | 315 | 354.6 | 164.8 |
| 181 | 203.8 | 92.6 | 226 | 254.4 | 116.4 | 271 | 305.1 | 140.6 | 316 | 355.8 | 165.3 |
| 182 | 204.9 | 93.1 | 227 | 255.6 | 116.9 | 272 | 306.2 | 141.1 | 317 | 356.9 | 165.9 |
| 183 | 206.0 | 93.7 | 228 | 256.7 | 117.4 | 273 | 307.3 | 141.7 | 318 | 358.0 | 166.4 |
| 184 | 207.1 | 94.2 | 229 | 257.8 | 118.0 | 274 | 308.5 | 142.2 | 319 | 359.1 | 167.0 |
| 185 | 208.3 | 94.7 | 230 | 258.9 | 118.5 | 275 | 309.6 | 142.8 | 320 | 360.3 | 167.5 |
| 186 | 209.4 | 95.2 | 231 | 260.1 | 119.0 | 276 | 310.7 | 143.3 | 321 | 361.4 | 168.1 |
| 187 | 210.5 | 95.7 | 232 | 261.2 | 119.6 | 277 | 311.9 | 143.9 | 322 | 362.5 | 168.6 |
| 188 | 211.7 | 96.3 | 233 | 262.3 | 120.1 | 278 | 313.0 | 144.4 | 323 | 363.7 | 169.2 |
| 189 | 212.8 | 96.8 | 234 | 263.4 | 120.7 | 279 | 314.1 | 145.0 | 324 | 364.8 | 169.7 |
| 190 | 213.9 | 97.3 | 235 | 264.6 | 121.2 | 280 | 315.2 | 145.5 | 325 | 365.9 | 170.3 |
| 191 | 215.0 | 97.8 | 236 | 265.7 | 121.7 | 281 | 316.4 | 146.1 | 326 | 367.0 | 170.9 |
| 192 | 216.2 | 98.4 | 237 | 266.8 | 122.3 | 282 | 317.5 | 146.6 | 327 | 368.2 | 171.4 |
| 193 | 217.3 | 98.9 | 238 | 268.0 | 122.8 | 283 | 318.6 | 147.2 | 328 | 369.3 | 172.0 |
| 194 | 218.4 | 99.4 | 239 | 269.1 | 123.4 | 284 | 319.7 | 147.7 | 329 | 370.4 | 172.5 |
| 195 | 219.5 | 100.0 | 240 | 270.2 | 123.9 | 285 | 320.9 | 148.3 | 330 | 371.5 | 173.1 |
| 196 | 220.7 | 100.5 | 241 | 271.3 | 124.4 | 286 | 322.0 | 148.8 | 331 | 372.7 | 173.7 |
| 197 | 221.8 | 101.0 | 242 | 272.5 | 125.0 | 287 | 323.1 | 149.4 | 332 | 373.8 | 174.2 |
| 198 | 222.9 | 101.5 | 243 | 273.6 | 125.5 | 288 | 324.2 | 149.9 | 333 | 374.9 | 174.8 |
| 199 | 224.0 | 102.0 | 244 | 274.7 | 126.0 | 289 | 325.4 | 150.5 | 334 | 376.0 | 175.3 |
| 200 | 225.2 | 102.6 | 245 | 275.8 | 126.6 | 290 | 326.5 | 151.0 | 335 | 377.2 | 175.9 |
| 201 | 226.3 | 103.1 | 246 | 277.0 | 127.1 | 291 | 327.4 | 151.6 | 336 | 378.3 | 176.5 |
| 202 | 227.4 | 103.7 | 247 | 278.1 | 127.6 | 292 | 328.7 | 152.1 | 337 | 379.4 | 177.0 |
| 203 | 228.5 | 104.2 | 248 | 279.2 | 128.1 | 293 | 329.9 | 152.7 | 338 | 380.5 | 177.6 |
| 204 | 229.7 | 104.7 | 249 | 280.3 | 128.7 | 294 | 331.0 | 153.2 | 339 | 381.7 | 178.1 |
| 205 | 230.8 | 105.3 | 250 | 281.5 | 129.2 | 295 | 332.1 | 153.8 | 340 | 382.8 | 178.7 |
| 206 | 231.9 | 105.8 | 251 | 282.6 | 129.7 | 296 | 333.3 | 154.3 | 341 | 383.9 | 179.3 |
| 207 | 233.0 | 106.3 | 252 | 283.7 | 130.3 | 297 | 334.4 | 154.9 | 342 | 385.0 | 179.8 |
| 208 | 234.2 | 106.8 | 253 | 284.8 | 130.8 | 298 | 335.5 | 155.4 | 343 | 386.2 | 180.4 |
| 209 | 235.3 | 107.4 | 254 | 286.0 | 131.4 | 299 | 336.6 | 156.0 | 344 | 387.3 | 180.9 |
| 210 | 236.4 | 107.9 | 255 | 287.1 | 131.9 | 300 | 337.8 | 156.5 | 345 | 388.4 | 181.5 |
| 211 | 237.6 | 108.4 | 256 | 288.2 | 132.4 | 301 | 338.9 | 157.1 | 346 | 389.6 | 182.1 |
| 212 | 238.7 | 109.0 | 257 | 289.3 | 133.0 | 302 | 340.0 | 157.6 | 347 | 390.7 | 182.6 |
| 213 | 239.8 | 109.5 | 258 | 290.5 | 133.5 | 303 | 341.1 | 158.2 | 348 | 391.8 | 183.2 |
| 214 | 240.9 | 110.0 | 259 | 291.6 | 134.1 | 304 | 342.3 | 158.7 | 349 | 392.9 | 183.7 |
| 215 | 242.1 | 110.6 | 260 | 292.7 | 134.6 | 305 | 343.4 | 159.3 | 350 | 394.0 | 184.3 |

TABLE 10. (*Concluded.*)

| Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu.) | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 351 | 395.2 | 184.9 | 380 | 427.8 | 201.4 | 408 | 459.4 | 217.5 | 436 | 490.9 | 233.9 |
| 352 | 396.3 | 185.4 | 381 | 429.0 | 202.0 | 409 | 460.5 | 218.1 | 437 | 492.0 | 234.5 |
| 353 | 397.4 | 186.0 | 382 | 430.1 | 202.5 | 410 | 461.6 | 218.7 | 438 | 493.1 | 235.1 |
| 354 | 398.6 | 186.6 | 383 | 431.2 | 203.1 | 411 | 462.7 | 219.3 | 439 | 494.3 | 235.7 |
| 355 | 399.7 | 187.2 | 384 | 432.3 | 203.7 | 412 | 463.8 | 219.9 | 440 | 495.4 | 236.3 |
| 356 | 400.8 | 187.7 | 385 | 433.5 | 204.3 | 413 | 465.0 | 220.4 | 441 | 496.5 | 236.9 |
| 357 | 401.9 | 188.3 | 386 | 434.6 | 204.8 | 414 | 466.1 | 221.0 | 442 | 497.6 | 237.5 |
| 358 | 403.1 | 188.9 | 387 | 435.7 | 205.4 | 415 | 467.2 | 221.6 | 443 | 498.8 | 238.1 |
| 359 | 404.2 | 189.4 | 388 | 436.8 | 206.0 | 416 | 468.4 | 222.2 | 444 | 499.9 | 238.7 |
| 360 | 405.3 | 190.0 | 389 | 438.0 | 206.5 | 417 | 469.5 | 222.8 | 445 | 501.0 | 239.3 |
| 361 | 406.4 | 190.6 | 390 | 439.1 | 207.1 | 418 | 470.6 | 223.3 | 446 | 502.1 | 239.8 |
| 362 | 407.6 | 191.1 | 391 | 440.2 | 207.7 | 419 | 471.8 | 223.9 | 447 | 503.2 | 240.4 |
| 363 | 408.7 | 191.7 | 392 | 441.3 | 208.3 | 420 | 472.9 | 224.5 | 448 | 504.4 | 241.0 |
| 364 | 409.8 | 192.3 | 393 | 442.4 | 208.8 | 421 | 474.0 | 225.1 | 449 | 505.5 | 241.6 |
| 365 | 410.9 | 192.9 | 394 | 443.6 | 209.4 | 422 | 475.6 | 225.7 | 450 | 506.6 | 242.2 |
| 366 | 412.1 | 193.4 | 395 | 444.7 | 210.0 | 423 | 476.2 | 226.3 | 451 | 507.8 | 242.8 |
| 367 | 413.2 | 194.0 | 396 | 445.9 | 210.6 | 424 | 477.4 | 226.9 | 452 | 508.9 | 243.4 |
| 368 | 414.3 | 194.6 | 397 | 447.0 | 211.2 | 425 | 478.5 | 227.5 | 453 | 510.0 | 244.0 |
| 369 | 415.4 | 195.1 | 398 | 448.1 | 211.7 | 426 | 479.6 | 228.0 | 454 | 511.1 | 244.6 |
| 370 | 416.6 | 195.7 | 399 | 449.2 | 212.3 | 427 | 480.7 | 228.6 | 455 | 512.3 | 245.2 |
| 371 | 417.7 | 196.3 | 400 | 450.3 | 212.9 | 428 | 481.9 | 229.2 | 456 | 513.4 | 245.7 |
| 372 | 418.8 | 196.8 | 401 | 451.5 | 213.5 | 429 | 483.0 | 229.8 | 457 | 514.5 | 246.3 |
| 373 | 420.0 | 197.4 | 402 | 452.6 | 214.1 | 430 | 484.1 | 230.4 | 458 | 515.6 | 246.9 |
| 374 | 421.1 | 198.0 | 403 | 453.7 | 214.6 | 431 | 485.3 | 231.0 | 459 | 516.8 | 247.5 |
| 375 | 422.2 | 198.6 | 404 | 454.8 | 215.2 | 432 | 486.4 | 231.6 | 460 | 517.9 | 248.1 |
| 376 | 423.3 | 199.1 | 405 | 456.0 | 215.8 | 433 | 487.5 | 232.2 | 461 | 519.0 | 248.7 |
| 377 | 424.5 | 199.7 | 406 | 457.1 | 216.4 | 434 | 488.6 | 232.8 | 462 | 520.1 | 249.3 |
| 378 | 425.6 | 200.3 | 407 | 458.2 | 217.0 | 435 | 489.7 | 233.4 | 463 | 521.3 | 249.9 |
| 379 | 426.7 | 200.8 | | | | | | | | | |

## TABLE* 11.

### Pflügers Table for Determining Glucose.

| Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). |
|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 12 | 32.8 | 36.8 | 64 | 139.4 | 157.0 | 116 | 244.0 | 274.7 |
| 13 | 34.9 | 39.2 | 65 | 141.4 | 159.3 | 117 | 246.0 | 276.9 |
| 14 | 37.0 | 41.6 | 66 | 143.4 | 161.6 | 118 | 248.0 | 279.2 |
| 15 | 39.1 | 43.9 | 67 | 145.5 | 163.9 | 119 | 250.0 | 281.4 |
| 16 | 41.2 | 46.3 | 68 | 147.5 | 166.2 | **120** | 252.0 | 283.6 |
| 17 | 43.3 | 48.7 | 69 | 149.6 | 168.5 | 121 | 253.9 | 285.9 |
| 18 | 45.4 | 51.0 | **70** | 151.6 | 170.8 | 122 | 255.9 | 288.1 |
| 19 | 47.5 | 53.4 | 71 | 153.6 | 173.0 | 123 | 257.8 | 290.3 |
| **20** | 49.6 | 55.8 | 72 | 155.7 | 175.3 | 124 | 259.8 | 292.6 |
| 21 | 51.7 | 58.1 | 73 | 157.7 | 177.6 | 125 | 261.8 | 294.8 |
| 22 | 53.8 | 60.5 | 74 | 159.8 | 179.9 | 126 | 263.7 | 296.9 |
| 23 | 55.9 | 62.9 | 75 | 161.8 | 182.2 | 127 | 265.6 | 299.0 |
| 24 | 58.0 | 65.2 | 76 | 163.8 | 184.5 | 128 | 267.5 | 301.2 |
| 25 | 60.1 | 67.6 | 77 | 165.8 | 186.7 | 129 | 269.3 | 303.3 |
| 26 | 62.1 | 69.9 | 78 | 167.9 | 189.0 | **130** | 271.2 | 305.4 |
| 27 | 64.2 | 72.2 | 79 | 169.9 | 191.3 | 131 | 273.1 | 307.5 |
| 28 | 66.2 | 74.5 | **80** | 171.9 | 193.6 | 132 | 275.0 | 309.6 |
| 29 | 68.2 | 76.8 | 81 | 173.9 | 195.8 | 133 | 276.9 | 311.8 |
| **30** | 70.2 | 79.1 | 82 | 175.9 | 198.1 | 134 | 278.8 | 313.9 |
| 31 | 72.3 | 81.3 | 83 | 178.0 | 200.4 | 135 | 280.6 | 316.0 |
| 32 | 74.3 | 83.6 | 84 | 180.0 | 202.6 | 136 | 282.5 | 318.1 |
| 33 | 76.3 | 85.9 | 85 | 182.0 | 204.9 | 137 | 284.4 | 320.2 |
| 34 | 78.4 | 88.2 | 86 | 184.0 | 207.2 | 138 | 286.3 | 322.4 |
| 35 | 80.4 | 90.5 | 87 | 186.0 | 209.5 | 139 | 288.2 | 324.5 |
| 36 | 82.4 | 92.8 | 88 | 188.1 | 211.7 | **140** | 290.1 | 326.6 |
| 37 | 84.4 | 95.1 | 89 | 190.1 | 214.0 | 141 | 291.9 | 328.7 |
| 38 | 86.5 | 97.4 | **90** | 192.1 | 216.3 | 142 | 293.8 | 330.8 |
| 39 | 88.5 | 99.7 | 91 | 194.1 | 218.6 | 143 | 295.7 | 333.0 |
| **40** | 90.5 | 101.9 | 92 | 196.1 | 220.8 | 144 | 297.6 | 335.1 |
| 41 | 92.6 | 104.2 | 93 | 198.2 | 223.1 | 145 | 299.5 | 337.2 |
| 42 | 94.6 | 106.5 | 94 | 200.2 | 225.4 | 146 | 301.4 | 339.3 |
| 43 | 96.6 | 108.8 | 95 | 202.2 | 227.6 | 147 | 303.2 | 341.4 |
| 44 | 98.6 | 111.1 | 96 | 204.2 | 229.9 | 148 | 305.1 | 343.6 |
| 45 | 100.7 | 113.4 | 97 | 206.2 | 232.2 | 149 | 307.0 | 345.7 |
| 46 | 102.7 | 115.7 | 98 | 208.3 | 234.5 | **150** | 308.9 | 347.8 |
| 47 | 104.7 | 118.0 | 99 | 210.3 | 236.7 | 151 | 310.7 | 349.8 |
| 48 | 106.7 | 120.2 | **100** | 212.3 | 239.0 | 152 | 312.4 | 351.8 |
| 49 | 108.8 | 122.5 | 101 | 214.3 | 241.2 | 153 | 314.2 | 353.8 |
| **50** | 110.8 | 124.8 | 102 | 216.3 | 243.5 | 154 | 315.9 | 355.7 |
| 51 | 112.8 | 127.1 | 103 | 218.2 | 245.7 | 155 | 317.7 | 357.7 |
| 52 | 114.9 | 129.4 | 104 | 220.2 | 247.9 | 156 | 319.5 | 359.7 |
| 53 | 116.9 | 131.7 | 105 | 222.2 | 250.2 | 157 | 321.2 | 361.7 |
| 54 | 119.0 | 134.0 | 106 | 224.2 | 252.4 | 158 | 323.0 | 363.7 |
| 55 | 121.0 | 136.3 | 107 | 226.2 | 254.6 | 159 | 324.7 | 365.7 |
| 56 | 123.0 | 138.6 | 108 | 228.1 | 256.8 | **160** | 326.5 | 367.7 |
| 57 | 125.1 | 140.9 | 109 | 230.1 | 259.1 | 161 | 328.3 | 369.6 |
| 58 | 127.1 | 143.2 | **110** | 232.1 | 261.3 | 162 | 330.0 | 371.6 |
| 59 | 129.2 | 145.5 | 111 | 234.1 | 263.6 | 163 | 331.8 | 373.6 |
| **60** | 131.2 | 147.8 | 112 | 236.1 | 265.8 | 164 | 333.5 | 375.6 |
| 61 | 133.2 | 150.1 | 113 | 238.0 | 268.0 | 165 | 335.3 | 377.6 |
| 62 | 135.3 | 152.4 | 114 | 240.0 | 270.2 | 166 | 337.1 | 379.6 |
| 63 | 137.3 | 154.7 | 115 | 242.0 | 272.5 | 167 | 338.8 | 381.6 |

* *See* "Handbook," page 419.*

TABLE 11. (*Concluded.*)

| Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). | Glucose. | Copper. (Cu). | Cuprous oxide. ($Cu_2O$). |
|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 168 | 340.6 | 383.5 | 196 | 387.8 | 436.8 | **224** | 432.2 | 487.0 |
| 169 | 342.3 | 385.5 | 197 | 389.5 | 438.7 | 225 | 433.8 | 488.8 |
| **170** | 344.1 | 387.5 | 198 | 391.2 | 440.6 | 226 | 435.3 | 490.4 |
| 171 | 345.9 | 389.5 | 199 | 392.8 | 442.4 | 227 | 436.7 | 492.1 |
| 172 | 347.6 | 391.5 | **200** | 394.5 | 444.3 | 228 | 438.1 | 493.7 |
| 173 | 349.4 | 393.5 | 201 | 396.1 | 446.1 | 229 | 439.6 | 495.3 |
| 174 | 351.1 | 395.5 | 202 | 397.6 | 447.9 | **230** | 441.1 | 497.0 |
| 175 | 352.9 | 397.5 | 203 | 399.2 | 449.6 | 231 | 442.6 | 498.6 |
| 176 | 354.6 | 399.3 | 204 | 400.8 | 451.4 | 232 | 444.0 | 500.3 |
| 177 | 356.2 | 401.2 | 205 | 402.4 | 453.2 | 233 | 445.5 | 501.9 |
| 178 | 357.9 | 403.1 | 206 | 403.9 | 455.0 | 234 | 446.9 | 503.5 |
| 179 | 359.6 | 404.9 | 207 | 405.5 | 456.8 | 235 | 448.4 | 505.2 |
| **180** | 361.2 | 406.8 | 208 | 407.1 | 458.5 | 236 | 449.9 | 506.8 |
| 181 | 362.9 | 408.7 | 209 | 408.6 | 460.3 | 237 | 451.3 | 508.4 |
| 182 | 364.5 | 410.6 | **210** | 410.2 | 462.1 | 238 | 452.8 | 510.1 |
| 183 | 366.2 | 412.4 | 211 | 411.8 | 463.9 | 239 | 454.2 | 511.7 |
| 184 | 367.9 | 414.3 | 212 | 413.4 | 465.7 | **240** | 455.7 | 513.3 |
| 185 | 369.5 | 416.2 | 213 | 414.9 | 467.4 | 241 | 457.2 | 515.0 |
| 186 | 371.2 | 418.1 | 214 | 416.5 | 469.2 | 242 | 458.6 | 516.6 |
| 187 | 372.9 | 419.9 | 215 | 418.1 | 471.0 | 243 | 460.1 | 518.2 |
| 188 | 374.5 | 421.8 | 216 | 419.7 | 472.8 | 244 | 461.5 | 519.9 |
| 189 | 376.2 | 423.7 | 217 | 421.2 | 474.6 | 245 | 463.0 | 521.5 |
| **190** | 377.9 | 425.6 | 218 | 422.8 | 476.3 | 246 | 464.5 | 523.6 |
| 191 | 379.5 | 427.4 | 219 | 424.4 | 478.1 | 247 | 465.9 | 524.8 |
| 192 | 381.2 | 429.3 | **220** | 425.9 | 479.9 | 248 | 467.4 | 526.4 |
| 193 | 382.9 | 431.2 | 221 | 427.5 | 481.7 | 249 | 468.8 | 528.1 |
| 194 | 384.5 | 433.1 | 222 | 429.1 | 483.5 | **250** | 470.3 | 529.7 |
| 195 | 386.2 | 434.9 | 223 | 430.7 | 485.2 | | | |

TABLE* 12.

KOCH AND RUHSAM'S TABLE FOR DETERMINING GLUCOSE IN TANNING MATERIALS.

| Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 1 | 0.4 | 53 | 22.8 | 105 | 49.5 | 157 | 75.5 |
| 2 | 0.8 | 54 | 23.3 | 106 | 50.0 | 158 | 76.0 |
| 3 | 1.2 | 55 | 23.9 | 107 | 50.5 | 159 | 76.5 |
| 4 | 1.6 | 56 | 24.4 | 108 | 51.0 | **160** | 77.0 |
| 5 | 2.0 | 57 | 24.9 | 109 | 51.6 | 161 | 77.5 |
| 6 | 2.5 | 58 | 25.4 | **110** | 52.1 | 162 | 78.0 |
| 7 | 2.9 | 59 | 25.9 | 111 | 52.6 | 163 | 78.5 |
| 8 | 3.3 | **60** | 26.4 | 112 | 53.1 | 164 | 79.0 |
| 9 | 3.7 | 61 | 26.9 | 113 | 53.6 | 165 | 79.5 |
| **10** | 4.1 | 62 | 27.4 | 114 | 54.1 | 166 | 80.0 |
| 11 | 4.5 | 63 | 28.0 | 115 | 54.6 | 167 | 80.5 |
| 12 | 4.9 | 64 | 28.5 | 116 | 55.1 | 168 | 81.0 |
| 13 | 5.3 | 65 | 29.0 | 117 | 55.7 | 169 | 81.4 |
| 14 | 5.7 | 66 | 29.5 | 118 | 56.2 | **170** | 81.9 |
| 15 | 6.1 | 67 | 30.0 | 119 | 56.7 | 171 | 82.4 |
| 16 | 6.5 | 68 | 30.5 | **120** | 57.2 | 172 | 82.9 |
| 17 | 7.0 | 69 | 31.0 | 121 | 57.7 | 173 | 83.4 |
| 18 | 7.4 | **70** | 31.6 | 122 | 58.2 | 174 | 83.9 |
| 19 | 7.8 | 71 | 32.1 | 123 | 58.7 | 175 | 84.4 |
| **20** | 8.2 | 72 | 32.6 | 124 | 59.2 | 176 | 84.9 |
| 21 | 8.6 | 73 | 33.1 | 125 | 59.7 | 177 | 85.4 |
| 22 | 9.0 | 74 | 33.6 | 126 | 60.2 | 178 | 85.9 |
| 23 | 9.4 | 75 | 34.1 | 127 | 60.7 | 179 | 86.4 |
| 24 | 9.9 | 76 | 34.6 | 128 | 61.2 | **180** | 86.9 |
| 25 | 10.3 | 77 | 35.1 | 129 | 61.7 | 181 | 87.4 |
| 26 | 10.7 | 78 | 35.7 | **130** | 62.2 | 182 | 87.9 |
| 27 | 11.1 | 79 | 36.2 | 131 | 62.6 | 183 | 88.4 |
| 28 | 11.6 | **80** | 36.7 | 132 | 63.1 | 184 | 88.9 |
| 29 | 12.0 | 81 | 37.2 | 133 | 63.6 | 185 | 89.4 |
| **30** | 12.4 | 82 | 37.7 | 134 | 64.1 | 186 | 89.9 |
| 31 | 12.9 | 83 | 38.2 | 135 | 64.6 | 187 | 90.4 |
| 32 | 13.3 | 84 | 38.7 | 136 | 65.1 | 188 | 90.9 |
| 33 | 13.7 | 85 | 39.2 | 137 | 65.6 | 189 | 91.3 |
| 34 | 14.1 | 86 | 39.8 | 138 | 66.1 | **190** | 91.8 |
| 35 | 14.6 | 87 | 40.3 | 139 | 66.6 | 191 | 92.3 |
| 36 | 15.0 | 88 | 40.8 | **140** | 67.1 | 192 | 92.8 |
| 37 | 15.4 | 89 | 41.2 | 141 | 67.6 | 193 | 93.3 |
| 38 | 15.9 | **90** | 41.8 | 142 | 68.1 | 194 | 93.8 |
| 39 | 16.3 | 91 | 42.3 | 143 | 68.6 | 195 | 94.3 |
| **40** | 16.7 | 92 | 42.8 | 144 | 69.1 | 196 | 94.8 |
| 41 | 17.2 | 93 | 43.3 | 145 | 69.6 | 197 | 95.3 |
| 42 | 17.6 | 94 | 43.9 | 146 | 70.1 | 198 | 95.8 |
| 43 | 18.0 | 95 | 44.4 | 147 | 70.6 | 199 | 96.3 |
| 44 | 18.4 | 96 | 44.9 | 148 | 71.1 | **200** | 96.8 |
| 45 | 18.9 | 97 | 45.4 | 149 | 71.5 | 201 | 97.3 |
| 46 | 19.3 | 98 | 45.9 | **150** | 72.0 | 202 | 97.8 |
| 47 | 19.7 | 99 | 46.4 | 151 | 72.5 | 203 | 98.3 |
| 48 | 20.2 | **100** | 46.9 | 152 | 73.0 | 204 | 98.8 |
| 49 | 20.7 | 101 | 47.5 | 153 | 73.5 | 205 | 99.3 |
| **50** | 21.3 | 102 | 48.0 | 154 | 74.0 | 206 | 99.8 |
| 51 | 21.8 | 103 | 48.5 | 155 | 74.5 | 207 | 100.3 |
| 52 | 22.3 | 104 | 49.0 | 156 | 75.0 | 208 | 100.8 |

* *See* "Handbook," page 420.

TABLE 12. (*Continued.*)

| Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 209 | 101.4 | 263 | 129.5 | 317 | 158.1 | 371 | 188.3 |
| **210** | 101.9 | 264 | 130.1 | 318 | 158.7 | 372 | 188.8 |
| 211 | 102.4 | 265 | 130.6 | 319 | 159.2 | 373 | 189.4 |
| 212 | 102.9 | 266 | 131.1 | **320** | 159.8 | 374 | 190.0 |
| 213 | 103.5 | 267 | 131.6 | 321 | 160.3 | 375 | 190.6 |
| 214 | 104.0 | 268 | 132.2 | 322 | 160.9 | 376 | 191.1 |
| 215 | 104.5 | 269 | 132.7 | 323 | 161.4 | 377 | 191.7 |
| 216 | 105.0 | **270** | 133.2 | 324 | 162.0 | 378 | 192.3 |
| 217 | 105.5 | 271 | 133.7 | 325 | 162.5 | 379 | 192.8 |
| 218 | 106.0 | 272 | 134.2 | 326 | 163.0 | **380** | 193.4 |
| 219 | 106.6 | 273 | 134.7 | 327 | 163.6 | 381 | 194.0 |
| **220** | 107.1 | 274 | 135.5 | 328 | 164.1 | 382 | 194.6 |
| 221 | 107.6 | 275 | 135.8 | 329 | 164.7 | 383 | 195.2 |
| 222 | 108.1 | 276 | 136.3 | **330** | 165.2 | 384 | 195.7 |
| 223 | 108.7 | 277 | 136.8 | 331 | 165.8 | 385 | 196.3 |
| 224 | 109.2 | 278 | 137.4 | 332 | 166.3 | 386 | 196.9 |
| 225 | 109.7 | 279 | 137.9 | 333 | 166.9 | 387 | 197.5 |
| 226 | 110.2 | **280** | 138.4 | 334 | 167.4 | 388 | 198.0 |
| 227 | 110.7 | 281 | 139.0 | 335 | 167.9 | 389 | 198.6 |
| 228 | 111.2 | 282 | 139.5 | 336 | 168.4 | **390** | 199.2 |
| 229 | 111.8 | 283 | 140.0 | 337 | 169.0 | 391 | 199.8 |
| **230** | 112.3 | 284 | 140.5 | 338 | 169.5 | 392 | 200.3 |
| 231 | 112.8 | 285 | 141.1 | 339 | 170.1 | 393 | 200.9 |
| 232 | 113.3 | 286 | 141.6 | **340** | 170.6 | 394 | 201.5 |
| 233 | 113.8 | 287 | 142.1 | 341 | 171.2 | 395 | 202.1 |
| 234 | 114.4 | 288 | 142.6 | 342 | 171.7 | 396 | 202.7 |
| 235 | 114.9 | 289 | 143.2 | 343 | 172.2 | 397 | 203.3 |
| 236 | 115.4 | **290** | 143.7 | 344 | 172.8 | 398 | 203.8 |
| 237 | 115.9 | 291 | 144.2 | 345 | 173.3 | 399 | 204.4 |
| 238 | 116.4 | 292 | 144.7 | 346 | 173.9 | **400** | 205.0 |
| 239 | 117.0 | 293 | 145.3 | 347 | 174.5 | 401 | 205.6 |
| **240** | 117.5 | 294 | 145.8 | 348 | 175.0 | 402 | 206.2 |
| 241 | 118.0 | 295 | 146.3 | 349 | 175.6 | 403 | 206.8 |
| 242 | 118.5 | 296 | 146.9 | **350** | 176.2 | 404 | 207.3 |
| 243 | 119.0 | 297 | 147.4 | 351 | 176.8 | 405 | 207.9 |
| 244 | 119.5 | 298 | 147.9 | 352 | 177.3 | 406 | 208.5 |
| 245 | 120.1 | 299 | 148.4 | 353 | 177.9 | 407 | 209.1 |
| 246 | 120.6 | **300** | 149.0 | 354 | 178.5 | 408 | 209.7 |
| 247 | 121.1 | 301 | 149.5 | 355 | 179.1 | 409 | 210.3 |
| 248 | 121.6 | 302 | 150.1 | 356 | 179.6 | **410** | 210.8 |
| 249 | 122.1 | 303 | 150.6 | 357 | 180.2 | 411 | 211.4 |
| **250** | 122.7 | 304 | 151.1 | 358 | 180.8 | 412 | 212.0 |
| 251 | 123.2 | 305 | 151.7 | 359 | 181.4 | 413 | 212.6 |
| 252 | 123.7 | 306 | 152.2 | **360** | 181.9 | 414 | 213.2 |
| 253 | 124.2 | 307 | 152.8 | 361 | 182.5 | 415 | 213.8 |
| 254 | 124.8 | 308 | 153.3 | 362 | 183.1 | 416 | 214.4 |
| 255 | 125.3 | 309 | 153.9 | 363 | 183.7 | 417 | 214.9 |
| 256 | 125.8 | **310** | 154.4 | 364 | 184.2 | 418 | 215.5 |
| 257 | 126.3 | 311 | 155.0 | 365 | 184.8 | 419 | 216.1 |
| 258 | 126.9 | 312 | 155.5 | 366 | 185.4 | **420** | 216.7 |
| 259 | 127.5 | 313 | 156.0 | 367 | 186.0 | 421 | 217.3 |
| **260** | 128.0 | 314 | 156.5 | 368 | 186.5 | 422 | 217.9 |
| 261 | 128.5 | 315 | 157.1 | 369 | 187.1 | 423 | 218.4 |
| 262 | 129.0 | 316 | 157.6 | **370** | 187.7 | 424 | 219.0 |

TABLE 12. (*Concluded.*)

| Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. | Copper. (Cu). | Glucose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 425 | 219.6 | 438 | 227.8 | 451 | 236.6 | 464 | 245.3 |
| 426 | 220.2 | 439 | 228.5 | 452 | 237.2 | 465 | 246.0 |
| 427 | 220.8 | **440** | 229.1 | 453 | 237.9 | 466 | 246.7 |
| 428 | 221.4 | 441 | 229.8 | 454 | 238.6 | 467 | 247.4 |
| 429 | 221.9 | 442 | 230.5 | 455 | 239.3 | 468 | 248.0 |
| **430** | 222.5 | 443 | 231.2 | 456 | 239.9 | 469 | 248.7 |
| 431 | 223.1 | 444 | 231.8 | 457 | 240.6 | **470** | 249.4 |
| 432 | 223.7 | 445 | 232.5 | 458 | 241.3 | 471 | 250.1 |
| 433 | 224.4 | 446 | 233.2 | 459 | 242.0 | 472 | 250.8 |
| 434 | 225.1 | 447 | 233.9 | **460** | 242.6 | 473 | 251.4 |
| 435 | 225.8 | 448 | 234.5 | 461 | 243.3 | 474 | 252.1 |
| 436 | 226.4 | 449 | 235.2 | 462 | 244.0 | 475 | 252.8 |
| 437 | 227.1 | **450** | 235.9 | 463 | 244.7 | 476 | 253.5 |

TABLE* 13.

MEISSL'S TABLE FOR DETERMINING INVERT SUGAR.

| Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 90 | 46.9 | 135 | 70.8 | 180 | 95.2 | 225 | 120.4 |
| 91 | 47.4 | 136 | 71.3 | 181 | 95.7 | 226 | 120.9 |
| 92 | 47.9 | 137 | 71.9 | 182 | 96.2 | 227 | 121.5 |
| 93 | 48.4 | 138 | 72.4 | 183 | 96.8 | 228 | 122.1 |
| 94 | 48.9 | 139 | 72.9 | 184 | 97.3 | 229 | 122.6 |
| 95 | 49.5 | 140 | 73.5 | 185 | 97.8 | 230 | 123.2 |
| 96 | 50.0 | 141 | 74.0 | 186 | 98.4 | 231 | 123.8 |
| 97 | 50.5 | 142 | 74.5 | 187 | 99.0 | 232 | 124.3 |
| 98 | 51.1 | 143 | 75.1 | 188 | 99.5 | 233 | 124.9 |
| 99 | 51.6 | 144 | 75.6 | 189 | 100.1 | 234 | 125.5 |
| 100 | 52.1 | 145 | 76.1 | 190 | 100.6 | 235 | 126.0 |
| 101 | 52.7 | 146 | 76.7 | 191 | 101.2 | 236 | 126.6 |
| 102 | 53.2 | 147 | 77.2 | 192 | 101.7 | 237 | 127.2 |
| 103 | 53.7 | 148 | 77.8 | 193 | 102.3 | 238 | 127.8 |
| 104 | 54.3 | 149 | 78.3 | 194 | 102.9 | 239 | 128.3 |
| 105 | 54.8 | 150 | 78.9 | 195 | 103.4 | 240 | 128.9 |
| 106 | 55.3 | 151 | 79.4 | 196 | 104.0 | 241 | 129.5 |
| 107 | 55.9 | 152 | 80.0 | 197 | 104.6 | 242 | 130.0 |
| 108 | 56.4 | 153 | 80.5 | 198 | 105.1 | 243 | 130.6 |
| 109 | 56.9 | 154 | 81.0 | 199 | 105.7 | 244 | 131.2 |
| 110 | 57.5 | 155 | 81.6 | 200 | 106.3 | 245 | 131.8 |
| 111 | 58.0 | 156 | 82.1 | 201 | 106.8 | 246 | 132.3 |
| 112 | 58.5 | 157 | 82.7 | 202 | 107.4 | 247 | 132.9 |
| 113 | 59.1 | 158 | 83.2 | 203 | 107.9 | 248 | 133.5 |
| 114 | 59.6 | 159 | 83.8 | 204 | 108.5 | 249 | 134.1 |
| 115 | 60.1 | 160 | 84.3 | 205 | 109.1 | 250 | 134.6 |
| 116 | 60.7 | 161 | 84.8 | 206 | 109.6 | 251 | 135.2 |
| 117 | 61.2 | 162 | 85.4 | 207 | 110.2 | 252 | 135.8 |
| 118 | 61.7 | 163 | 85.9 | 208 | 110.8 | 253 | 136.3 |
| 119 | 62.3 | 164 | 86.5 | 209 | 111.3 | 254 | 136.9 |
| 120 | 62.8 | 165 | 87.0 | 210 | 111.9 | 255 | 137.5 |
| 121 | 63.3 | 166 | 87.6 | 211 | 112.5 | 256 | 138.1 |
| 122 | 63.9 | 167 | 88.1 | 212 | 113.0 | 257 | 138.6 |
| 123 | 64.4 | 168 | 88.6 | 213 | 113.6 | 258 | 139.2 |
| 124 | 64.9 | 169 | 89.2 | 214 | 114.2 | 259 | 139.8 |
| 125 | 65.5 | 170 | 89.7 | 215 | 114.7 | 260 | 140.4 |
| 126 | 66.0 | 171 | 90.3 | 216 | 115.3 | 261 | 140.9 |
| 127 | 66.5 | 172 | 90.8 | 217 | 115.8 | 262 | 141.5 |
| 128 | 67.1 | 173 | 91.4 | 218 | 116.4 | 263 | 142.1 |
| 129 | 67.6 | 174 | 91.9 | 219 | 117.0 | 264 | 142.7 |
| 130 | 68.1 | 175 | 92.4 | 220 | 117.5 | 265 | 143.2 |
| 131 | 68.7 | 176 | 93.0 | 221 | 118.1 | 266 | 143.8 |
| 132 | 69.2 | 177 | 93.5 | 222 | 118.7 | 267 | 144.4 |
| 133 | 69.7 | 178 | 94.1 | 223 | 119.2 | 268 | 144.9 |
| 134 | 70.3 | 179 | 94.6 | 224 | 119.8 | 269 | 145.5 |

* *See* "Handbook," page 423.

TABLE 13. (*Concluded.*)

| Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 270 | 146.1 | 310 | 169.7 | 350 | 193.8 | 390 | 218.7 |
| 271 | 146.7 | 311 | 170.3 | 351 | 194.4 | 391 | 219.3 |
| 272 | 147.2 | 312 | 170.9 | 352 | 195.0 | 392 | 219.9 |
| 273 | 147.8 | 313 | 171.5 | 353 | 195.6 | 393 | 220.5 |
| 274 | 148.4 | 314 | 172.1 | 354 | 196.2 | 394 | 221.2 |
| 275 | 149.0 | 315 | 172.7 | 355 | 196.8 | 395 | 221.8 |
| 276 | 149.5 | 316 | 173.3 | 356 | 197.4 | 396 | 222.4 |
| 277 | 150.1 | 317 | 173.9 | 357 | 198.0 | 397 | 223.1 |
| 278 | 150.7 | 318 | 174.5 | 358 | 198.6 | 398 | 223.7 |
| 279 | 151.3 | 319 | 175.1 | 359 | 199.2 | 399 | 224.3 |
| 280 | 151.9 | 320 | 175.6 | 360 | 199.8 | 400 | 224.9 |
| 281 | 152.5 | 321 | 176.2 | 361 | 200.4 | 401 | 225.7 |
| 282 | 153.1 | 322 | 176.8 | 362 | 201.1 | 402 | 226.4 |
| 283 | 153.7 | 323 | 177.4 | 363 | 201.7 | 403 | 227.1 |
| 284 | 154.3 | 324 | 178.0 | 364 | 202.3 | 404 | 227.8 |
| 285 | 154.9 | 325 | 178.6 | 365 | 203.0 | 405 | 228.6 |
| 286 | 155.5 | 326 | 179.2 | 366 | 203.6 | 406 | 229.3 |
| 287 | 156.1 | 327 | 179.8 | 367 | 204.2 | 407 | 230.0 |
| 288 | 156.7 | 328 | 180.4 | 368 | 204.8 | 408 | 230.7 |
| 289 | 157.2 | 329 | 181.0 | 369 | 205.5 | 409 | 231.4 |
| 290 | 157.8 | 330 | 181.6 | 370 | 206.1 | 410 | 232.1 |
| 291 | 158.4 | 331 | 182.2 | 371 | 206.7 | 411 | 232.8 |
| 292 | 159.0 | 332 | 182.8 | 372 | 207.3 | 412 | 233.5 |
| 293 | 159.6 | 333 | 183.5 | 373 | 208.0 | 413 | 234.3 |
| 294 | 160.2 | 334 | 184.1 | 374 | 208.6 | 414 | 235.0 |
| 295 | 160.8 | 335 | 184.7 | 375 | 209.2 | 415 | 235.7 |
| 296 | 161.4 | 336 | 185.4 | 376 | 209.9 | 416 | 236.4 |
| 297 | 162.0 | 337 | 186.0 | 377 | 210.5 | 417 | 237.1 |
| 298 | 162.6 | 338 | 186.6 | 378 | 211.1 | 418 | 237.8 |
| 299 | 163.2 | 339 | 187.2 | 379 | 211.7 | 419 | 238.5 |
| 300 | 163.8 | 340 | 187.8 | 380 | 212.4 | 420 | 239.2 |
| 301 | 164.4 | 341 | 188.4 | 381 | 213.0 | 421 | 239.9 |
| 302 | 165.0 | 342 | 189.0 | 382 | 213.6 | 422 | 240.6 |
| 303 | 165.6 | 343 | 189.6 | 383 | 214.3 | 423 | 241.3 |
| 304 | 166.2 | 344 | 190.2 | 384 | 214.9 | 424 | 242.0 |
| 305 | 166.8 | 345 | 190.8 | 385 | 215.5 | 425 | 242.7 |
| 306 | 167.3 | 346 | 191.4 | 386 | 216.1 | 426 | 243.4 |
| 307 | 167.9 | 347 | 192.0 | 387 | 216.8 | 427 | 244.1 |
| 308 | 168.5 | 348 | 192.6 | 388 | 217.4 | 428 | 244.9 |
| 309 | 169.1 | 349 | 193.2 | 389 | 218.0 | 429 | 245.6 |
| | | | | | | 430 | 246.3 |

TABLE* 14.

WEIN'S TABLE FOR DETERMINING MALTOSE.

| Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 31 | 34.9 | 26.1 | 76 | 85.6 | 65.4 | 121 | 136.2 | 105.3 | 166 | 186.9 | 145.8 |
| 32 | 36.0 | 27.0 | 77 | 86.7 | 66.2 | 122 | 137.4 | 106.2 | 167 | 188.0 | 146.7 |
| 33 | 37.2 | 27.9 | 78 | 87.8 | 67.1 | 123 | 138.5 | 107.1 | 168 | 189.1 | 147.6 |
| 34 | 38.3 | 28.7 | 79 | 88.9 | 68.0 | 124 | 139.6 | 108.0 | 169 | 190.3 | 148.5 |
| 35 | 39.4 | 29.6 | 80 | 90.1 | 68.9 | 125 | 140.7 | 108.9 | 170 | 191.4 | 149.4 |
| 36 | 40.5 | 30.5 | 81 | 91.2 | 69.7 | 126 | 141.9 | 109.8 | 171 | 192.5 | 150.3 |
| 37 | 41.7 | 31.3 | 82 | 92.3 | 70.6 | 127 | 143.0 | 110.7 | 172 | 193.6 | 151.2 |
| 38 | 42.8 | 32.2 | 83 | 93.4 | 71.5 | 128 | 144.1 | 111.6 | 173 | 194.8 | 152.0 |
| 39 | 43.9 | 33.1 | 84 | 94.6 | 72.4 | 129 | 145.2 | 112.5 | 174 | 195.9 | 152.9 |
| 40 | 45.0 | 33.9 | 85 | 95.7 | 73.2 | 130 | 146.4 | 113.4 | 175 | 197.0 | 153.8 |
| 41 | 46.2 | 34.8 | 86 | 96.8 | 74.1 | 131 | 147.5 | 114.3 | 176 | 198.1 | 154.7 |
| 42 | 47.3 | 35.7 | 87 | 97.9 | 75.0 | 132 | 148.6 | 115.2 | 177 | 199.3 | 155.6 |
| 43 | 48.4 | 36.5 | 88 | 99.1 | 75.9 | 133 | 149.7 | 116.1 | 178 | 200.4 | 156.5 |
| 44 | 49.5 | 37.4 | 89 | 100.2 | 76.8 | 134 | 150.9 | 117.0 | 179 | 201.5 | 157.4 |
| 45 | 50.7 | 38.3 | 90 | 101.3 | 77.7 | 135 | 152.0 | 117.9 | 180 | 202.6 | 158.3 |
| 46 | 51.8 | 39.1 | 91 | 102.4 | 78.6 | 136 | 153.1 | 118.8 | 181 | 203.8 | 159.2 |
| 47 | 52.9 | 40.0 | 92 | 103.6 | 79.5 | 137 | 154.2 | 119.7 | 182 | 204.9 | 160.1 |
| 48 | 54.0 | 40.9 | 93 | 104.7 | 80.3 | 138 | 155.4 | 120.6 | 183 | 206.0 | 160.9 |
| 49 | 55.2 | 41.8 | 94 | 105.8 | 81.2 | 139 | 156.5 | 121.5 | 184 | 207.1 | 161.8 |
| 50 | 56.3 | 42.6 | 95 | 107.0 | 82.1 | 140 | 157.6 | 122.4 | 185 | 208.3 | 162.7 |
| 51 | 57.4 | 43.5 | 96 | 108.1 | 83.0 | 141 | 158.7 | 123.3 | 186 | 209.4 | 163.6 |
| 52 | 58.5 | 44.4 | 97 | 109.2 | 83.9 | 142 | 159.9 | 124.2 | 187 | 210.5 | 164.5 |
| 53 | 59.7 | 45.2 | 98 | 110.3 | 84.8 | 143 | 161.0 | 125.1 | 188 | 211.7 | 165.4 |
| 54 | 60.8 | 46.1 | 99 | 111.5 | 85.7 | 144 | 162.1 | 126.0 | 189 | 212.8 | 166.3 |
| 55 | 61.9 | 47.0 | 100 | 112.6 | 86.6 | 145 | 163.2 | 126.9 | 190 | 213.9 | 167.2 |
| 56 | 63.0 | 47.8 | 101 | 113.7 | 87.5 | 146 | 164.4 | 127.8 | 191 | 215.0 | 168.1 |
| 57 | 64.2 | 48.7 | 102 | 114.8 | 88.4 | 147 | 165.5 | 128.7 | 192 | 216.2 | 169.0 |
| 58 | 65.3 | 49.6 | 103 | 116.0 | 89.2 | 148 | 166.6 | 129.6 | 193 | 217.3 | 169.8 |
| 59 | 66.4 | 50.4 | 104 | 117.1 | 90.1 | 149 | 167.7 | 130.5 | 194 | 218.4 | 170.7 |
| 60 | 67.6 | 51.3 | 105 | 118.2 | 91.0 | 150 | 168.9 | 131.4 | 195 | 219.5 | 171.6 |
| 61 | 68.7 | 52.2 | 106 | 119.3 | 91.9 | 151 | 170.0 | 132.3 | 196 | 220.7 | 172.5 |
| 62 | 69.8 | 53.1 | 107 | 120.5 | 92.8 | 152 | 171.1 | 133.2 | 197 | 221.8 | 173.4 |
| 63 | 70.9 | 53.9 | 108 | 121.6 | 93.7 | 153 | 172.3 | 134.1 | 198 | 222.9 | 174.3 |
| 64 | 72.1 | 54.8 | 109 | 122.7 | 94.6 | 154 | 173.4 | 135.0 | 199 | 224.0 | 175.2 |
| 65 | 73.2 | 55.7 | 110 | 123.8 | 95.5 | 155 | 174.5 | 135.9 | 200 | 225.2 | 176.1 |
| 66 | 74.3 | 56.6 | 111 | 125.0 | 96.4 | 156 | 175.6 | 136.8 | 201 | 226.3 | 177.0 |
| 67 | 75.4 | 57.4 | 112 | 126.1 | 97.3 | 157 | 176.8 | 137.7 | 202 | 227.4 | 177.9 |
| 68 | 76.6 | 58.3 | 113 | 127.2 | 98.1 | 158 | 177.9 | 138.6 | 203 | 228.5 | 178.7 |
| 69 | 77.7 | 59.2 | 114 | 128.3 | 99.0 | 159 | 179.0 | 139.5 | 204 | 229.7 | 179.6 |
| 70 | 78.8 | 60.1 | 115 | 129.6 | 99.9 | 160 | 180.1 | 140.4 | 205 | 230.8 | 180.5 |
| 71 | 79.9 | 61.0 | 116 | 130.6 | 100.8 | 161 | 181.3 | 141.3 | 206 | 231.9 | 181.4 |
| 72 | 81.1 | 61.8 | 117 | 131.7 | 101.7 | 162 | 182.4 | 142.2 | 207 | 233.0 | 182.3 |
| 73 | 82.2 | 62.7 | 118 | 132.8 | 102.6 | 163 | 183.5 | 143.1 | 208 | 234.2 | 183.2 |
| 74 | 83.3 | 63.6 | 119 | 134.0 | 103.5 | 164 | 184.6 | 144.0 | 209 | 235.3 | 184.1 |
| 75 | 84.4 | 64.5 | 120 | 135.1 | 104.4 | 165 | 185.8 | 144.9 | 210 | 236.4 | 185.0 |

* *See* "Handbook," page 423.

TABLE 14. (*Concluded.*)

| Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. | Copper (Cu). | Cuprous oxide ($Cu_2O$). | Maltose. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 211 | 237.6 | 185.9 | 236 | 265.7 | 208.3 | 261 | 293.8 | 230.7 | 286 | 322.0 | 253.1 |
| 212 | 238.7 | 186.8 | 237 | 266.8 | 209.1 | 262 | 295.0 | 231.6 | 287 | 323.1 | 254.0 |
| 213 | 239.8 | 187.7 | 238 | 268.0 | 210.0 | 263 | 296.1 | 232.5 | 288 | 324.2 | 254.9 |
| 214 | 240.9 | 188.6 | 239 | 269.1 | 210.9 | 264 | 297.2 | 233.4 | 289 | 325.4 | 255.8 |
| 215 | 242.1 | 189.5 | 240 | 270.2 | 211.8 | 265 | 298.3 | 234.3 | 290 | 326.5 | 256.6 |
| 216 | 243.2 | 190.4 | 241 | 271.3 | 212.7 | 266 | 299.5 | 235.2 | 291 | 327.4 | 257.5 |
| 217 | 244.3 | 191.2 | 242 | 272.5 | 213.6 | 267 | 300.6 | 236.1 | 292 | 328.7 | 258.4 |
| 218 | 245.4 | 192.1 | 243 | 273.6 | 214.5 | 268 | 301.7 | 237.0 | 293 | 329.9 | 259.3 |
| 219 | 246.6 | 193.0 | 244 | 274.7 | 215.4 | 269 | 302.8 | 237.9 | 294 | 331.0 | 260.2 |
| 220 | 247.7 | 193.9 | 245 | 275.8 | 216.3 | 270 | 304.0 | 238.8 | 295 | 332.1 | 261.1 |
| 221 | 248.7 | 194.8 | 246 | 277.0 | 217.2 | 271 | 305.1 | 239.7 | 296 | 333.2 | 262.0 |
| 222 | 249.9 | 195.7 | 247 | 278.1 | 218.1 | 272 | 306.2 | 240.6 | 297 | 334.4 | 262.8 |
| 223 | 251.0 | 196.6 | 248 | 279.2 | 219.0 | 273 | 307.3 | 241.5 | 298 | 335.5 | 263.7 |
| 224 | 252.4 | 197.5 | 249 | 280.3 | 219.9 | 274 | 308.5 | 242.4 | 299 | 336.6 | 264.6 |
| 225 | 253.3 | 198.4 | 250 | 281.5 | 220.8 | 275 | 309.6 | 243.3 | 300 | 337.8 | 265.5 |
| 226 | 254.4 | 199.3 | 251 | 282.6 | 221.7 | 276 | 310.7 | 244.2 | | | |
| 227 | 255.6 | 200.2 | 252 | 283.7 | 222.6 | 277 | 311.9 | 245.1 | | | |
| 228 | 256.7 | 201.1 | 253 | 284.8 | 223.5 | 278 | 313.0 | 246.0 | | | |
| 229 | 257.8 | 202.0 | 254 | 286.0 | 224.4 | 279 | 314.1 | 246.9 | | | |
| 230 | 258.9 | 202.9 | 255 | 287.1 | 225.3 | 280 | 315.2 | 247.8 | | | |
| 231 | 260.1 | 203.8 | 256 | 288.2 | 226.2 | 281 | 316.4 | 248.7 | | | |
| 232 | 261.2 | 204.7 | 257 | 289.3 | 227.1 | 282 | 317.5 | 249.6 | | | |
| 233 | 262.3 | 205.6 | 258 | 290.5 | 228.0 | 283 | 318.6 | 250.4 | | | |
| 234 | 263.4 | 206.5 | 259 | 291.6 | 228.9 | 284 | 319.7 | 251.3 | | | |
| 235 | 264.6 | 207.4 | 260 | 292.7 | 229.8 | 285 | 320.9 | 252.2 | | | |

TABLE* 15.

SOXHLET AND WEIN'S TABLE FOR DETERMINING LACTOSE.

| Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. |
|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 100 | 71.6 | 145 | 105.1 | 190 | 139.3 | 235 | 173.1 | 280 | 208.3 |
| 101 | 72.4 | 146 | 105.8 | 191 | 140.0 | 236 | 173.9 | 281 | 209.1 |
| 102 | 73.1 | 147 | 106.6 | 192 | 140.8 | 237 | 174.6 | 282 | 209.9 |
| 103 | 73.8 | 148 | 107.3 | 193 | 141.6 | 238 | 175.4 | 283 | 210.7 |
| 104 | 74.6 | 149 | 108.1 | 194 | 142.3 | 239 | 176.2 | 284 | 211.5 |
| 105 | 75.3 | 150 | 108.8 | 195 | 143.1 | 240 | 176.9 | 285 | 212.3 |
| 106 | 76.1 | 151 | 109.6 | 196 | 143.9 | 241 | 177.7 | 286 | 213.1 |
| 107 | 76.8 | 152 | 110.3 | 197 | 144.6 | 242 | 178.5 | 287 | 213.9 |
| 108 | 77.6 | 153 | 111.1 | 198 | 145.4 | 243 | 179.3 | 288 | 214.7 |
| 109 | 78.3 | 154 | 111.9 | 199 | 146.2 | 244 | 180.1 | 289 | 215.5 |
| 110 | 79.0 | 155 | 112.6 | 200 | 146.9 | 245 | 180.8 | 290 | 216.3 |
| 111 | 79.8 | 156 | 113.4 | 201 | 147.7 | 246 | 181.6 | 291 | 217.1 |
| 112 | 80.5 | 157 | 114.1 | 202 | 148.5 | 247 | 182.4 | 292 | 217.9 |
| 113 | 81.3 | 158 | 114.9 | 203 | 149.2 | 248 | 183.2 | 293 | 218.7 |
| 114 | 82.0 | 159 | 115.6 | 204 | 150.0 | 249 | 184.0 | 294 | 219.5 |
| 115 | 82.7 | 160 | 116.4 | 205 | 150.7 | 250 | 184.8 | 295 | 220.3 |
| 116 | 83.5 | 161 | 117.1 | 206 | 151.5 | 251 | 185.5 | 296 | 221.1 |
| 117 | 84.2 | 162 | 117.9 | 207 | 152.2 | 252 | 186.3 | 297 | 221.9 |
| 118 | 85.0 | 163 | 118.6 | 208 | 153.0 | 253 | 187.1 | 298 | 222.7 |
| 119 | 85.7 | 164 | 119.4 | 209 | 153.7 | 254 | 187.9 | 299 | 223.5 |
| 120 | 86.4 | 165 | 120.2 | 210 | 154.5 | 255 | 188.7 | 300 | 224.4 |
| 121 | 87.2 | 166 | 120.9 | 211 | 155.2 | 256 | 189.4 | 301 | 225.2 |
| 122 | 87.9 | 167 | 121.7 | 212 | 156.0 | 257 | 190.2 | 302 | 225.9 |
| 123 | 88.7 | 168 | 122.4 | 213 | 156.7 | 258 | 191.0 | 303 | 226.7 |
| 124 | 89.4 | 169 | 123.2 | 214 | 157.5 | 259 | 191.8 | 304 | 227.5 |
| 125 | 90.1 | 170 | 123.9 | 215 | 158.2 | 260 | 192.5 | 305 | 228.3 |
| 126 | 90.9 | 171 | 124.7 | 216 | 159.0 | 261 | 193.3 | 306 | 229.1 |
| 127 | 91.6 | 172 | 125.5 | 217 | 159.7 | 262 | 194.1 | 307 | 229.8 |
| 128 | 92.4 | 173 | 126.2 | 218 | 160.4 | 263 | 194.9 | 308 | 230.6 |
| 129 | 93.1 | 174 | 127.0 | 219 | 161.2 | 264 | 195.7 | 309 | 231.4 |
| 130 | 93.8 | 175 | 127.8 | 220 | 161.9 | 265 | 196.4 | 310 | 232.2 |
| 131 | 94.6 | 176 | 128.5 | 221 | 162.7 | 266 | 197.2 | 311 | 232.9 |
| 132 | 95.3 | 177 | 129.3 | 222 | 163.4 | 267 | 198.0 | 312 | 233.7 |
| 133 | 96.1 | 178 | 130.1 | 223 | 164.2 | 268 | 198.8 | 313 | 234.5 |
| 134 | 96.9 | 179 | 130.8 | 224 | 164.9 | 269 | 199.5 | 314 | 235.3 |
| 135 | 97.6 | 180 | 131.6 | 225 | 165.7 | 270 | 200.3 | 315 | 236.1 |
| 136 | 98.3 | 181 | 132.4 | 226 | 166.4 | 271 | 201.1 | 316 | 236.8 |
| 137 | 99.1 | 182 | 133.1 | 227 | 167.2 | 272 | 201.9 | 317 | 237.6 |
| 138 | 99.8 | 183 | 133.9 | 228 | 167.9 | 273 | 202.7 | 318 | 238.4 |
| 139 | 100.5 | 184 | 134.7 | 229 | 168.6 | 274 | 203.5 | 319 | 239.2 |
| 140 | 101.3 | 185 | 135.4 | 230 | 169.4 | 275 | 204.3 | 320 | 240.0 |
| 141 | 102.0 | 186 | 136.2 | 231 | 170.1 | 276 | 205.1 | 321 | 240.7 |
| 142 | 102.8 | 187 | 137.0 | 232 | 170.9 | 277 | 205.9 | 322 | 241.5 |
| 143 | 103.5 | 188 | 137.7 | 233 | 171.6 | 278 | 206.7 | 323 | 242.3 |
| 144 | 104.3 | 189 | 138.5 | 234 | 172.4 | 279 | 207.5 | 324 | 243.1 |

* *See* "Handbook," page 424.

TABLE 15. (*Concluded.*)

| Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. | Copper. (Cu). | Lactose. |
|---|---|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 325 | 243.9 | 341 | 256.5 | 356 | 268.8 | 371 | 281.4 | 386 | 294.2 |
| 326 | 244.6 | 342 | 257.4 | 357 | 269.6 | 372 | 282.2 | 387 | 295.1 |
| 327 | 245.4 | 343 | 258.2 | 358 | 270.4 | 373 | 283.1 | 388 | 296.0 |
| 328 | 246.2 | 344 | 259.0 | 359 | 271.2 | 374 | 283.9 | 389 | 296.8 |
| 329 | 247.0 | 345 | 259.8 | 360 | 272.1 | 375 | 284.8 | 390 | 297.7 |
| 330 | 247.7 | 346 | 260.6 | 361 | 272.9 | 376 | 285.7 | 391 | 298.5 |
| 331 | 248.5 | 347 | 261.4 | 362 | 273.7 | 377 | 286.5 | 392 | 299.4 |
| 332 | 249.2 | 348 | 262.3 | 363 | 274.5 | 378 | 287.4 | 393 | 300.3 |
| 333 | 250.0 | 349 | 263.1 | 364 | 275.3 | 379 | 288.2 | 394 | 301.1 |
| 334 | 250.8 | 350 | 263.9 | 365 | 276.2 | 380 | 289.1 | 395 | 302.0 |
| 335 | 251.6 | 351 | 264.7 | 366 | 277.1 | 381 | 289.9 | 396 | 302.8 |
| 336 | 252.5 | 352 | 265.5 | 367 | 277.9 | 382 | 290.8 | 397 | 303.7 |
| 337 | 253.3 | 353 | 266.3 | 368 | 278.8 | 383 | 291.7 | 398 | 304.6 |
| 338 | 254.1 | 354 | 267.2 | 369 | 279.6 | 384 | 292.5 | 399 | 305.4 |
| 339 | 254.9 | 355 | 268.0 | 370 | 280.5 | 385 | 293.4 | 400 | 306.3 |
| 340 | 255.7 | | | | | | | | |

## TABLE* 16.

### Woy's Table for Determining Glucose, Fructose, Invert Sugar, Lactose and Maltose by Kjeldahl's Method.

15 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 5 | 4.0 | 1.7 | 2.1 | 2.0 | 2.0 | 2.8 | 3.0 |
| 6 | 4.8 | 2.1 | 2.5 | 2.4 | 2.4 | 3.3 | 3.6 |
| 7 | 5.6 | 2.5 | 2.9 | 2.8 | 2.8 | 3.8 | 4.2 |
| 8 | 6.4 | 2.9 | 3.3 | 3.2 | 3.2 | 4.3 | 4.8 |
| 9 | 7.2 | 3.2 | 3.7 | 3.6 | 3.6 | 4.8 | 5.4 |
| 10 | 8.0 | 3.5 | 4.1 | 4.0 | 4.0 | 5.4 | 6.0 |
| 11 | 8.8 | 3.9 | 4.5 | 4.4 | 4.4 | 5.9 | 6.6 |
| 12 | 9.6 | 4.2 | 5.0 | 4.9 | 4.9 | 6.4 | 7.2 |
| 13 | 10.4 | 4.6 | 5.4 | 5.3 | 5.2 | 7.0 | 7.8 |
| 14 | 11.2 | 5.0 | 5.8 | 5.7 | 5.7 | 7.5 | 8.4 |
| 15 | 12.0 | 5.4 | 6.2 | 6.1 | 6.1 | 8.1 | 9.0 |
| 16 | 12.8 | 5.7 | 6.6 | 6.4 | 6.5 | 8.7 | 9.6 |
| 17 | 13.6 | 6.1 | 7.0 | 6.8 | 6.9 | 9.2 | 10.2 |
| 18 | 14.4 | 6.5 | 7.4 | 7.2 | 7.3 | 9.8 | 10.8 |
| 19 | 15.2 | 6.8 | 7.9 | 7.6 | 7.7 | 10.3 | 11.4 |
| 20 | 16.0 | 7.2 | 8.3 | 8.0 | 8.1 | 10.8 | 12.0 |
| 21 | 16.8 | 7.6 | 8.7 | 8.4 | 8.6 | 11.4 | 12.6 |
| 22 | 17.6 | 7.9 | 9.2 | 8.8 | 9.0 | 11.9 | 13.2 |
| 23 | 18.4 | 8.3 | 9.6 | 9.2 | 9.4 | 12.5 | 13.8 |
| 24 | 19.2 | 8.7 | 10.0 | 9.6 | 9.8 | 13.0 | 14.5 |
| 25 | 20.0 | 9.0 | 10.4 | 10.0 | 10.2 | 13.6 | 15.1 |
| 26 | 20.8 | 9.4 | 10.8 | 10.4 | 10.6 | 14.2 | 15.7 |
| 27 | 21.6 | 9.8 | 11.3 | 10.8 | 11.1 | 14.7 | 16.3 |
| 28 | 22.4 | 10.1 | 11.7 | 11.2 | 11.5 | 15.2 | 16.9 |
| 29 | 23.2 | 10.5 | 12.1 | 11.6 | 11.9 | 15.8 | 17.5 |
| 30 | 24.0 | 10.9 | 12.5 | 12.0 | 12.3 | 16.4 | 18.1 |
| 31 | 24.8 | 11.2 | 13.0 | 12.4 | 12.8 | 17.0 | 18.8 |
| 32 | 25.6 | 11.6 | 13.4 | 12.8 | 13.2 | 17.5 | 19.4 |
| 33 | 26.4 | 12.0 | 13.8 | 13.2 | 13.6 | 18.0 | 20.0 |
| 34 | 27.2 | 12.4 | 14.2 | 13.6 | 14.0 | 18.6 | 20.6 |
| 35 | 28.0 | 12.8 | 14.7 | 14.0 | 14.4 | 19.1 | 21.2 |
| 36 | 28.7 | 13.2 | 15.1 | 14.4 | 14.9 | 19.7 | 21.8 |
| 37 | 29.5 | 13.5 | 15.5 | 14.8 | 15.3 | 20.2 | 22.4 |
| 38 | 30.3 | 13.9 | 16.0 | 15.2 | 15.7 | 20.7 | 23.1 |
| 39 | 31.1 | 14.3 | 16.4 | 15.5 | 16.1 | 21.3 | 23.7 |
| 40 | 31.9 | 14.6 | 16.8 | 16.0 | 16.5 | 21.8 | 24.3 |
| 41 | 32.7 | 15.0 | 17.3 | 16.4 | 16.9 | 22.4 | 24.9 |
| 42 | 33.5 | 15.4 | 17.7 | 16.8 | 17.4 | 22.9 | 25.5 |
| 43 | 34.3 | 15.8 | 18.1 | 17.2 | 17.8 | 23.5 | 26.1 |
| 44 | 35.1 | 16.1 | 18.5 | 17.6 | 18.2 | 24.1 | 26.7 |
| 45 | 35.9 | 16.5 | 18.9 | 18.0 | 18.6 | 24.7 | 27.4 |
| 46 | 36.7 | 17.0 | 19.4 | 18.5 | 19.1 | 25.3 | 28.1 |
| 47 | 37.5 | 17.4 | 19.8 | 18.9 | 19.6 | 25.9 | 28.7 |
| 48 | 38.3 | 17.8 | 20.3 | 19.3 | 20.0 | 26.4 | 29.3 |
| 49 | 39.1 | 18.2 | 20.9 | 19.7 | 20.5 | 27.0 | 30.0 |

* *See* "Handbook," page 424.

TABLE 16. (*Continued.*)
15 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert. sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 50 | 39.9 | 18.6 | 21.2 | 20.2 | 20.9 | 27.6 | 30.7 |
| 51 | 40.7 | 19.0 | 21.6 | 20.6 | 21.3 | 28.1 | 31.3 |
| 52 | 41.5 | 19.4 | 22.0 | 21.0 | 21.8 | 28.7 | 31.9 |
| 53 | 42.3 | 19.8 | 22.5 | 21.4 | 22.2 | 29.3 | 32.5 |
| 54 | 43.1 | 20.2 | 22.9 | 21.8 | 22.7 | 29.9 | 33.2 |
| 55 | 43.9 | 20.6 | 23.4 | 22.3 | 23.2 | 30.5 | 33.9 |
| 56 | 44.7 | 21.0 | 23.8 | 22.7 | 23.6 | 31.1 | 34.5 |
| 57 | 45.5 | 21.4 | 24.2 | 23.1 | 24.0 | 31.7 | 35.1 |
| 58 | 46.3 | 21.8 | 24.7 | 23.5 | 24.5 | 32.2 | 35.7 |
| 59 | 47.1 | 22.2 | 25.2 | 24.0 | 24.9 | 32.8 | 36.4 |
| 60 | 47.9 | 22.7 | 25.6 | 24.4 | 25.4 | 33.4 | 37.1 |
| 61 | 48.7 | 23.1 | 26.0 | 24.9 | 25.9 | 34.0 | 37.7 |
| 62 | 49.5 | 23.5 | 26.5 | 25.3 | 26.3 | 34.6 | 38.3 |
| 63 | 50.3 | 23.9 | 27.0 | 25.7 | 26.8 | 35.1 | 39.0 |
| 64 | 51.1 | 24.3 | 27.4 | 26.1 | 27.2 | 35.7 | 39.6 |
| 65 | 51.9 | 24.7 | 27.9 | 26.6 | 27.7 | 36.4 | 40.3 |
| 66 | 52.7 | 25.1 | 28.3 | 27.0 | 28.1 | 36.9 | 40.9 |
| 67 | 53.5 | 25.5 | 28.7 | 27.4 | 28.6 | 37.5 | 41.5 |
| 68 | 54.3 | 25.9 | 29.1 | 27.8 | 29.0 | 38.1 | 42.2 |
| 69 | 55.1 | 26.3 | 29.6 | 28.2 | 29.5 | 38.7 | 42.9 |
| 70 | 55.9 | 26.8 | 30.1 | 28.7 | 30.0 | 39.3 | 43.6 |
| 71 | 56.7 | 27.2 | 30.5 | 29.1 | 30.4 | 39.8 | 44.2 |
| 72 | 57.5 | 27.6 | 31.0 | 29.6 | 30.9 | 40.4 | 44.8 |
| 73 | 58.3 | 28.0 | 31.4 | 30.0 | 31.4 | 40.9 | 45.4 |
| 74 | 59.1 | 28.5 | 31.9 | 30.5 | 31.8 | 41.6 | 46.1 |
| 75 | 59.9 | 28.9 | 32.4 | 30.9 | 32.3 | 42.2 | 46.9 |
| 76 | 60.7 | 29.3 | 32.9 | 31.3 | 32.7 | 42.8 | 47.5 |
| 77 | 61.5 | 29.7 | 33.2 | 31.7 | 33.2 | 43.4 | 48.1 |
| 78 | 62.3 | 30.2 | 33.7 | 32.2 | 33.7 | 43.9 | 48.7 |
| 79 | 63.1 | 30.6 | 34.2 | 32.7 | 34.2 | 44.6 | 49.4 |
| 80 | 63.9 | 31.0 | 34.7 | 33.1 | 34.7 | 45.2 | 50.2 |
| 81 | 64.7 | 31.4 | 35.1 | 33.5 | 35.1 | 45.8 | 50.8 |
| 82 | 65.5 | 31.9 | 35.5 | 34.0 | 35.6 | 46.4 | 51.4 |
| 83 | 66.3 | 32.3 | 36.0 | 34.5 | 36.1 | 47.0 | 52.1 |
| 84 | 67.1 | 32.8 | 36.5 | 34.9 | 36.6 | 47.6 | 52.8 |
| 85 | 67.9 | 33.2 | 37.0 | 35.4 | 37.1 | 48.2 | 53.5 |
| 86 | 68.7 | 33.6 | 37.4 | 35.8 | 37.5 | 48.8 | 54.1 |
| 87 | 69.5 | 34.1 | 37.9 | 36.3 | 38.0 | 49.4 | 54.8 |
| 88 | 70.3 | 34.5 | 38.3 | 36.7 | 38.5 | 50.0 | 55.4 |
| 89 | 71.1 | 35.0 | 38.8 | 37.2 | 39.0 | 50.6 | 56.1 |
| 90 | 71.9 | 35.4 | 39.3 | 37.6 | 39.5 | 51.3 | 56.9 |
| 91 | 72.7 | 35.8 | 39.7 | 38.1 | 39.9 | 51.8 | 57.5 |
| 92 | 73.5 | 36.3 | 40.2 | 38.5 | 40.4 | 52.4 | 58.2 |
| 93 | 74.3 | 36.8 | 40.7 | 39.0 | 40.9 | 53.0 | 58.8 |
| 94 | 75.1 | 37.3 | 41.2 | 39.5 | 41.4 | 53.7 | 59.5 |
| 95 | 75.9 | 37.7 | 41.7 | 39.9 | 42.0 | 54.4 | 60.3 |
| 96 | 76.7 | 38.1 | 42.0 | 40.3 | 42.4 | 54.9 | 60.9 |
| 97 | 77.5 | 38.6 | 42.5 | 40.8 | 42.9 | 55.6 | 61.6 |
| 98 | 78.3 | 39.1 | 43.0 | 41.3 | 43.4 | 56.1 | 62.4 |
| 99 | 79.1 | 39.5 | 43.5 | 41.8 | 43.9 | 56.8 | 63.0 |

TABLE 16. (*Continued.*)
15 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 100 | 79.9 | 40.0 | 44.0 | 42.3 | 44.4 | 57.5 | 63.8 |
| 101 | 80.7 | 40.4 | 44.4 | 42.7 | 44.8 | 58.1 | 64.4 |
| 102 | 81.5 | 40.9 | 44.9 | 43.1 | 45.3 | 58.7 | 65.0 |
| 103 | 82.3 | 41.4 | 45.4 | 43.7 | 45.8 | 59.3 | 65.7 |
| 104 | 83.1 | 41.9 | 45.9 | 44.2 | 46.5 | 60.0 | 66.5 |
| 105 | 83.9 | 42.4 | 46.4 | 44.7 | 47.0 | 60.7 | 67.2 |
| 106 | 84.7 | 42.8 | 46.8 | 45.1 | 47.4 | 61.3 | 67.8 |
| 107 | 85.5 | 43.3 | 47.3 | 45.6 | 47.8 | 61.9 | 68.5 |
| 108 | 86.3 | 43.8 | 47.8 | 46.1 | 48.5 | 62.4 | 69.2 |
| 109 | 87.1 | 44.3 | 48.3 | 46.6 | 49.0 | 63.1 | 69.9 |
| 110 | 87.8 | 44.7 | 48.7 | 47.0 | 49.5 | 63.6 | 70.5 |
| 111 | 88.6 | 45.1 | 49.2 | 47.5 | 50.0 | 64.3 | 71.2 |
| 112 | 89.4 | 45.6 | 49.7 | 48.0 | 50.5 | 65.0 | 72.0 |
| 113 | 90.2 | 46.1 | 50.1 | 48.4 | 50.9 | 65.6 | 72.6 |
| 114 | 91.0 | 46.6 | 50.6 | 48.9 | 51.5 | 66.2 | 73.3 |
| 115 | 91.8 | 47.1 | 51.2 | 49.4 | 52.1 | 66.8 | 74.0 |
| 116 | 92.6 | 47.6 | 51.7 | 49.9 | 52.6 | 67.5 | 74.7 |
| 117 | 93.4 | 48.1 | 52.1 | 50.4 | 53.1 | 68.1 | 75.5 |
| 118 | 94.2 | 48.6 | 52.6 | 50.9 | 53.6 | 68.8 | 76.2 |
| 119 | 95.0 | 49.1 | 53.1 | 51.4 | 54.2 | 69.5 | 76.9 |
| 120 | 95.8 | 49.6 | 53.6 | 51.9 | 54.7 | 69.1 | 77.6 |
| 121 | 96.6 | 50.1 | 54.1 | 52.4 | 55.2 | 70.8 | 78.3 |
| 122 | 97.4 | 50.6 | 54.6 | 52.9 | 55.7 | 71.4 | 79.0 |
| 123 | 98.2 | 51.1 | 55.1 | 53.4 | 56.3 | 72.1 | 79.7 |
| 124 | 99.0 | 51.6 | 55.6 | 53.9 | 56.8 | 72.7 | 80.4 |
| 125 | 99.8 | 52.2 | 56.1 | 54.4 | 57.4 | 73.4 | 81.2 |
| 126 | 100.6 | 52.7 | 56.6 | 54.9 | 57.9 | 74.0 | 81.8 |
| 127 | 101.4 | 53.2 | 57.0 | 55.4 | 58.5 | 74.7 | 82.6 |
| 128 | 102.2 | 53.7 | 57.5 | 55.9 | 59.0 | 75.4 | 83.4 |
| 129 | 103.0 | 54.2 | 58.1 | 56.4 | 59.6 | 76.0 | 84.1 |
| 130 | 103.8 | 54.8 | 58.6 | 57.0 | 60.2 | 76.7 | 84.9 |
| 131 | 104.6 | 55.3 | 59.1 | 57.5 | 60.7 | 77.3 | 85.5 |
| 132 | 105.4 | 55.8 | 59.6 | 58.0 | 61.3 | 78.0 | 86.3 |
| 133 | 106.2 | 56.3 | 60.0 | 58.4 | 61.8 | 78.7 | 87.0 |
| 134 | 107.0 | 56.9 | 60.6 | 59.0 | 62.4 | 79.3 | 87.7 |
| 135 | 107.8 | 57.5 | 61.1 | 59.6 | 63.0 | 79.9 | 88.4 |
| 136 | 108.6 | 58.0 | 61.6 | 60.1 | 63.5 | 80.6 | 89.0 |
| 137 | 109.4 | 58.5 | 62.1 | 60.6 | 64.0 | 81.3 | 89.8 |
| 138 | 110.2 | 59.0 | 62.6 | 61.1 | 64.5 | 82.0 | 90.6 |
| 139 | 111.0 | 59.6 | 63.1 | 61.6 | 65.2 | 82.7 | 91.4 |
| 140 | 111.8 | 60.2 | 63.7 | 62.2 | 65.8 | 83.3 | 92.1 |
| 141 | 112.6 | 60.7 | 64.2 | 62.7 | 66.3 | 84.0 | 92.8 |
| 142 | 113.4 | 61.3 | 64.7 | 63.3 | 66.9 | 84.7 | 93.6 |
| 143 | 114.2 | 61.8 | 65.1 | 63.7 | 67.5 | 85.4 | 94.4 |
| 144 | 115.0 | 62.4 | 65.7 | 64.3 | 68.1 | 86.1 | 95.1 |
| 145 | 115.8 | 63.0 | 66.2 | 64.9 | 68.7 | 86.7 | 95.9 |
| 146 | 116.6 | 63.5 | 66.7 | 65.4 | 69.2 | 87.4 | 96.6 |
| 147 | 117.4 | 64.1 | 67.3 | 66.0 | 69.8 | 88.1 | 97.4 |
| 148 | 118.2 | 64.7 | 67.8 | 66.5 | 70.4 | 88.8 | 98.1 |
| 149 | 119.0 | 65.3 | 68.3 | 67.1 | 71.0 | 89.5 | 98.9 |

TABLE 16. (*Continued.*)

15 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 150 | 119.8 | 65.8 | 68.9 | 67.7 | 71.6 | 90.1 | 99.6 |
| 151 | 120.6 | 66.5 | 69.4 | 68.2 | 72.1 | 90.8 | 100.4 |
| 152 | 121.4 | 67.1 | 70.0 | 68.9 | 72.9 | 91.5 | 101.2 |
| 153 | 122.2 | 67.6 | 70.4 | 69.3 | 73.4 | 92.3 | 101.9 |
| 154 | 123.0 | 68.3 | 70.9 | 69.9 | 74.0 | 93.0 | 102.7 |
| 155 | 123.8 | 68.9 | 71.5 | 70.5 | 74.7 | 93.7 | 103.4 |
| 156 | 124.6 | 69.5 | 72.0 | 71.0 | 75.3 | 94.4 | 104.2 |
| 157 | 125.4 | 70.1 | 72.6 | 71.6 | 75.9 | 95.1 | 105.0 |
| 158 | 126.2 | 70.7 | 73.0 | 72.1 | 76.4 | 95.8 | 105.7 |
| 159 | 127.0 | 71.3 | 73.6 | 72.7 | 77.1 | 96.5 | 106.5 |
| 160 | 127.8 | 72.0 | 74.2 | 73.4 | 77.7 | 97.2 | 107.2 |

30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 50 | 39.9 | 17.7 | 19.8 | 19.1 | 19.8 | 26.6 | 30.8 |
| 51 | 40.7 | 18.1 | 20.2 | 19.4 | 20.2 | 27.2 | 31.5 |
| 52 | 41.5 | 18.5 | 20.6 | 19.8 | 20.7 | 27.8 | 32.1 |
| 53 | 42.3 | 18.8 | 21.0 | 20.2 | 21.1 | 28.3 | 32.7 |
| 54 | 43.1 | 19.2 | 21.4 | 20.6 | 21.5 | 28.9 | 33.4 |
| 55 | 43.9 | 19.6 | 21.8 | 21.0 | 21.9 | 29.4 | 34.0 |
| 56 | 44.7 | 20.0 | 22.2 | 21.4 | 22.3 | 30.0 | 34.7 |
| 57 | 45.5 | 20.3 | 22.7 | 21.8 | 22.7 | 30.5 | 35.3 |
| 58 | 46.3 | 20.7 | 23.1 | 22.1 | 23.1 | 31.0 | 35.8 |
| 59 | 47.1 | 21.1 | 23.5 | 22.6 | 23.5 | 31.6 | 36.5 |
| 60 | 47.9 | 21.5 | 23.9 | 23.0 | 23.9 | 32.1 | 37.1 |
| 61 | 48.7 | 21.8 | 24.3 | 23.3 | 24.3 | 32.7 | 37.8 |
| 62 | 49.5 | 22.2 | 24.7 | 23.7 | 24.7 | 33.3 | 38.4 |
| 63 | 50.3 | 22.5 | 25.1 | 24.1 | 25.2 | 33.8 | 38.9 |
| 64 | 51.1 | 22.9 | 25.5 | 24.5 | 25.6 | 34.3 | 39.6 |
| 65 | 51.9 | 23.3 | 25.9 | 24.9 | 26.0 | 34.9 | 40.3 |
| 66 | 52.7 | 23.7 | 26.3 | 25.3 | 26.4 | 35.5 | 41.0 |
| 67 | 53.5 | 24.0 | 26.8 | 25.7 | 26.8 | 36.1 | 41.6 |
| 68 | 54.3 | 24.4 | 27.2 | 26.1 | 27.2 | 36.6 | 42.2 |
| 69 | 55.1 | 24.8 | 27.6 | 26.4 | 27.6 | 37.2 | 42.9 |
| 70 | 55.9 | 25.2 | 28.0 | 26.9 | 28.1 | 37.7 | 43.5 |
| 71 | 56.7 | 25.6 | 28.4 | 27.3 | 28.5 | 38.3 | 44.2 |
| 72 | 57.5 | 25.9 | 28.8 | 27.6 | 28.9 | 38.9 | 44.8 |
| 73 | 58.3 | 26.3 | 29.2 | 28.0 | 29.3 | 39.4 | 45.4 |
| 74 | 59.1 | 26.7 | 29.6 | 28.4 | 29.6 | 40.0 | 46.1 |
| 75 | 59.9 | 27.0 | 30.1 | 28.8 | 30.1 | 40.5 | 46.7 |
| 76 | 60.7 | 27.4 | 30.5 | 29.2 | 30.5 | 41.1 | 47.3 |
| 77 | 61.5 | 27.8 | 30.9 | 29.6 | 30.9 | 41.7 | 48.0 |
| 78 | 62.3 | 28.2 | 31.3 | 30.0 | 31.4 | 42.2 | 48.6 |
| 79 | 63.1 | 28.5 | 31.7 | 30.4 | 31.8 | 42.8 | 49.3 |

TABLE 16. (*Continued.*)
30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose. $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 80 | 63.9 | 28.9 | 32.1 | 30.8 | 32.2 | 43.3 | 49.9 |
| 81 | 64.7 | 29.3 | 32.5 | 31.2 | 32.6 | 43.9 | 50.6 |
| 82 | 65.5 | 29.7 | 32.9 | 31.6 | 33.0 | 44.5 | 51.2 |
| 83 | 66.3 | 30.1 | 33.4 | 32.0 | 33.5 | 45.0 | 51.8 |
| 84 | 67.1 | 30.4 | 33.8 | 32.4 | 33.9 | 45.6 | 52.5 |
| 85 | 67.9 | 30.8 | 34.2 | 32.8 | 34.3 | 46.1 | 53.1 |
| 86 | 68.7 | 31.2 | 34.6 | 33.2 | 34.9 | 46.7 | 53.8 |
| 87 | 69.5 | 31.6 | 35.0 | 33.6 | 35.1 | 47.3 | 54.4 |
| 88 | 70.3 | 32.0 | 35.4 | 34.0 | 35.6 | 47.8 | 55.0 |
| 89 | 71.1 | 32.3 | 35.9 | 34.4 | 36.0 | 48.4 | 55.7 |
| 90 | 71.9 | 32.7 | 36.3 | 34.8 | 36.4 | 48.9 | 56.3 |
| 91 | 72.7 | 33.1 | 36.7 | 35.2 | 36.8 | 49.5 | 57.0 |
| 92 | 73.5 | 33.5 | 37.1 | 35.6 | 37.2 | 50.1 | 57.7 |
| 93 | 74.3 | 33.9 | 37.6 | 36.0 | 37.7 | 50.6 | 58.3 |
| 94 | 75.1 | 34.3 | 38.0 | 36.4 | 38.1 | 51.2 | 59.0 |
| 95 | 75.9 | 34.6 | 38.4 | 36.8 | 38.5 | 51.7 | 59.6 |
| 96 | 76.7 | 35.0 | 38.8 | 37.2 | 38.9 | 52.3 | 60.3 |
| 97 | 77.5 | 35.4 | 39.3 | 37.6 | 39.4 | 52.9 | 60.9 |
| 98 | 78.3 | 35.8 | 39.7 | 38.0 | 39.8 | 53.4 | 61.5 |
| 99 | 79.1 | 36.2 | 40.1 | 38.4 | 40.2 | 54.0 | 62.2 |
| 100 | 79.9 | 36.6 | 40.5 | 38.8 | 40.7 | 54.5 | 62.9 |
| 101 | 80.7 | 37.0 | 40.9 | 39.2 | 41.1 | 55.1 | 63.6 |
| 102 | 81.5 | 37.4 | 41.4 | 39.7 | 41.5 | 55.7 | 64.2 |
| 103 | 82.3 | 37.7 | 41.8 | 40.2 | 41.9 | 56.2 | 64.8 |
| 104 | 83.1 | 38.1 | 42.2 | 40.5 | 42.4 | 56.8 | 65.4 |
| 105 | 83.9 | 38.5 | 42.7 | 40.9 | 42.8 | 57.4 | 66.1 |
| 106 | 84.7 | 38.9 | 43.1 | 41.3 | 43.2 | 58.0 | 66.8 |
| 107 | 85.5 | 39.3 | 43.5 | 41.7 | 43.6 | 58.6 | 67.5 |
| 108 | 86.3 | 39.7 | 44.0 | 42.1 | 44.1 | 59.1 | 68.1 |
| 109 | 87.1 | 40.1 | 44.4 | 42.5 | 44.5 | 59.7 | 68.8 |
| 110 | 87.8 | 40.4 | 44.7 | 42.8 | 44.8 | 60.2 | 69.3 |
| 111 | 88.6 | 40.7 | 45.2 | 43.2 | 45.3 | 60.8 | 70.0 |
| 112 | 89.4 | 41.1 | 45.6 | 43.6 | 45.8 | 61.4 | 70.8 |
| 113 | 90.2 | 41.5 | 46.0 | 44.0 | 46.1 | 61.9 | 71.4 |
| 114 | 91.0 | 41.9 | 46.5 | 44.4 | 46.6 | 62.5 | 72.0 |
| 115 | 91.8 | 42.3 | 46.9 | 44.9 | 47.0 | 63.1 | 72.7 |
| 116 | 92.6 | 42.7 | 47.2 | 45.3 | 47.4 | 63.7 | 73.3 |
| 117 | 93.4 | 43.1 | 47.7 | 45.7 | 47.9 | 64.3 | 74.0 |
| 118 | 94.2 | 43.5 | 48.2 | 46.1 | 48.3 | 64.8 | 74.6 |
| 119 | 95.0 | 43.9 | 48.6 | 46.5 | 48.7 | 65.4 | 75.3 |
| 120 | 95.8 | 44.3 | 49.0 | 46.9 | 49.2 | 66.0 | 75.9 |
| 121 | 96.6 | 44.7 | 49.5 | 47.4 | 49.6 | 66.5 | 76.7 |
| 122 | 97.4 | 45.1 | 49.9 | 47.8 | 50.0 | 67.1 | 77.3 |
| 123 | 98.2 | 45.5 | 50.3 | 48.2 | 50.5 | 67.7 | 78.0 |
| 124 | 99.0 | 45.9 | 50.8 | 48.6 | 50.9 | 68.3 | 78.6 |
| 125 | 99.8 | 46.3 | 51.2 | 49.0 | 51.4 | 68.9 | 79.3 |
| 126 | 100.6 | 46.7 | 51.7 | 49.5 | 51.8 | 69.4 | 80.0 |
| 127 | 101.4 | 47.1 | 52.1 | 49.9 | 52.2 | 70.0 | 80.6 |
| 128 | 102.2 | 47.5 | 52.5 | 50.3 | 52.7 | 70.6 | 81.3 |
| 129 | 103.0 | 47.9 | 53.0 | 50.7 | 53.1 | 71.2 | 81.9 |

TABLE 16. (*Continued.*)
30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert. sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 130 | 103.8 | 48.3 | 53.4 | 51.1 | 53.6 | 71.9 | 82.7 |
| 131 | 104.6 | 48.7 | 53.9 | 51.6 | 54.0 | 72.4 | 83.3 |
| 132 | 105.4 | 49.1 | 54.3 | 52.0 | 54.4 | 73.0 | 83.9 |
| 133 | 106.2 | 49.5 | 54.7 | 52.4 | 54.9 | 73.6 | 84.6 |
| 134 | 107.0 | 49.9 | 55.2 | 52.8 | 55.3 | 74.3 | 85.2 |
| 135 | 107.8 | 50.3 | 55.6 | 53.2 | 55.8 | 74.8 | 86.0 |
| 136 | 108.6 | 50.7 | 56.1 | 53.7 | 56.2 | 75.4 | 86.6 |
| 137 | 109.4 | 51.2 | 56.5 | 54.1 | 56.6 | 76.0 | 87.2 |
| 138 | 110.2 | 51.5 | 56.9 | 54.5 | 57.1 | 76.6 | 87.8 |
| 139 | 111.0 | 51.9 | 57.4 | 54.9 | 57.5 | 77.1 | 88.6 |
| 140 | 111.8 | 52.4 | 57.9 | 55.4 | 58.0 | 77.8 | 89.3 |
| 141 | 112.6 | 52.8 | 58.3 | 55.8 | 58.5 | 78.3 | 89.9 |
| 142 | 113.4 | 53.2 | 58.7 | 56.2 | 58.9 | 78.9 | 90.6 |
| 143 | 114.2 | 53.6 | 59.2 | 56.7 | 59.3 | 79.5 | 91.3 |
| 144 | 115.0 | 54.0 | 59.6 | 57.1 | 59.8 | 80.1 | 91.9 |
| 145 | 115.8 | 54.4 | 60.1 | 57.5 | 60.2 | 80.7 | 92.6 |
| 146 | 116.6 | 54.8 | 60.5 | 57.9 | 60.6 | 81.3 | 93.3 |
| 147 | 117.4 | 55.2 | 60.9 | 58.3 | 61.1 | 81.9 | 94.0 |
| 148 | 118.2 | 55.6 | 61.4 | 58.8 | 61.6 | 82.5 | 94.7 |
| 149 | 119.0 | 56.0 | 61.8 | 59.2 | 62.0 | 83.1 | 95.3 |
| 150 | 119.8 | 56.5 | 62.3 | 59.7 | 62.5 | 83.7 | 95.9 |
| 151 | 120.6 | 56.9 | 62.8 | 60.1 | 62.9 | 84.3 | 96.6 |
| 152 | 121.4 | 57.3 | 63.2 | 60.5 | 63.3 | 84.9 | 97.3 |
| 153 | 122.2 | 57.7 | 63.6 | 60.9 | 63.8 | 85.5 | 98.0 |
| 154 | 123.0 | 58.1 | 64.1 | 61.4 | 64.3 | 86.1 | 98.7 |
| 155 | 123.8 | 58.5 | 64.5 | 61.8 | 64.7 | 86.7 | 99.3 |
| 156 | 124.6 | 59.0 | 65.0 | 62.3 | 65.2 | 87.3 | 99.9 |
| 157 | 125.4 | 59.4 | 65.4 | 62.7 | 65.6 | 87.9 | 100.7 |
| 158 | 126.2 | 59.8 | 65.9 | 63.1 | 66.1 | 88.5 | 101.5 |
| 159 | 127.0 | 60.2 | 66.3 | 63.5 | 66.5 | 89.1 | 102.1 |
| 160 | 127.8 | 60.6 | 66.8 | 64.0 | 67.0 | 89.7 | 102.8 |
| 161 | 128.6 | 61.0 | 67.3 | 64.4 | 67.5 | 90.3 | 103.5 |
| 162 | 129.4 | 61.4 | 67.7 | 64.8 | 67.9 | 90.9 | 104.2 |
| 163 | 130.2 | 61.9 | 68.1 | 65.2 | 68.4 | 91.5 | 104.9 |
| 164 | 131.0 | 62.3 | 68.6 | 65.7 | 68.8 | 92.1 | 105.5 |
| 165 | 131.8 | 62.7 | 69.1 | 66.2 | 69.3 | 92.7 | 106.2 |
| 166 | 132.6 | 63.2 | 69.6 | 66.7 | 69.8 | 93.2 | 107.0 |
| 167 | 133.4 | 63.6 | 70.0 | 67.1 | 70.2 | 93.9 | 107.6 |
| 168 | 134.2 | 64.0 | 70.4 | 67.5 | 70.7 | 94.5 | 108.3 |
| 169 | 135.0 | 64.4 | 70.9 | 67.9 | 71.1 | 95.1 | 109.0 |
| 170 | 135.8 | 64.8 | 71.4 | 68.4 | 71.6 | 95.8 | 109.7 |
| 171 | 136.6 | 65.3 | 71.8 | 68.8 | 72.1 | 96.3 | 110.3 |
| 172 | 137.4 | 65.7 | 72.2 | 69.2 | 72.5 | 96.9 | 111.1 |
| 173 | 138.2 | 66.1 | 72.7 | 69.7 | 73.0 | 97.5 | 111.8 |
| 174 | 139.0 | 66.6 | 73.2 | 70.2 | 73.4 | 98.1 | 112.4 |
| 175 | 139.8 | 67.0 | 73.6 | 70.6 | 74.0 | 98.8 | 113.1 |
| 176 | 140.6 | 67.4 | 74.1 | 71.0 | 74.4 | 99.4 | 113.8 |
| 177 | 141.4 | 67.8 | 74.5 | 71.4 | 74.9 | 100.0 | 114.5 |
| 178 | 142.2 | 68.3 | 75.0 | 71.9 | 75.3 | 100.6 | 115.2 |
| 179 | 143.0 | 68.7 | 75.5 | 72.4 | 75.8 | 101.2 | 115.9 |

TABLE 16. (*Continued.*)
30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 180 | 143.8 | 69.1 | 76.0 | 72.8 | 76.3 | 101.8 | 116.5 |
| 181 | 144.6 | 69.6 | 76.4 | 73.3 | 76.7 | 102.4 | 117.2 |
| 182 | 145.4 | 70.0 | 76.8 | 73.7 | 77.1 | 103.0 | 118.0 |
| 183 | 146.2 | 70.4 | 77.3 | 74.1 | 77.6 | 103.6 | 118.7 |
| 184 | 147.0 | 70.9 | 77.8 | 74.6 | 78.1 | 104.2 | 119.3 |
| 185 | 147.7 | 71.3 | 78.2 | 75.0 | 78.5 | 104.8 | 119.9 |
| 186 | 148.5 | 71.7 | 78.7 | 75.5 | 79.0 | 105.4 | 120.7 |
| 187 | 149.3 | 72.2 | 79.2 | 76.0 | 79.5 | 105.9 | 121.3 |
| 188 | 150.1 | 72.6 | 79.7 | 76.4 | 80.0 | 106.6 | 122.0 |
| 189 | 150.9 | 73.0 | 80.1 | 76.8 | 80.5 | 107.3 | 122.8 |
| 190 | 151.7 | 73.4 | 80.5 | 77.2 | 80.9 | 107.9 | 123.4 |
| 191 | 152.5 | 73.9 | 81.0 | 77.7 | 81.4 | 108.5 | 124.2 |
| 192 | 153.3 | 74.3 | 81.5 | 78.2 | 81.8 | 109.0 | 124.8 |
| 193 | 154.1 | 74.8 | 82.0 | 78.7 | 82.3 | 109.7 | 125.5 |
| 194 | 154.9 | 75.2 | 82.5 | 79.1 | 82.8 | 110.3 | 126.2 |
| 195 | 155.7 | 75.6 | 82.9 | 79.5 | 83.2 | 111.0 | 126.9 |
| 196 | 156.5 | 76.1 | 83.4 | 80.0 | 83.7 | 111.6 | 127.7 |
| 197 | 157.3 | 76.6 | 83.9 | 80.5 | 84.2 | 112.2 | 128.4 |
| 198 | 158.1 | 77.0 | 84.4 | 81.0 | 84.7 | 112.8 | 129.1 |
| 199 | 158.9 | 77.5 | 84.9 | 81.5 | 85.2 | 113.4 | 129.8 |
| 200 | 159.7 | 77.9 | 85.3 | 81.9 | 85.6 | 114.1 | 130.5 |
| 201 | 160.5 | 78.3 | 85.8 | 82.3 | 86.1 | 114.7 | 131.2 |
| 202 | 161.3 | 78.8 | 86.3 | 82.8 | 86.6 | 115.3 | 131.9 |
| 203 | 162.1 | 79.3 | 86.8 | 83.3 | 87.1 | 116.0 | 132.6 |
| 204 | 162.9 | 79.7 | 87.3 | 83.8 | 87.6 | 116.5 | 133.3 |
| 205 | 163.7 | 80.1 | 87.7 | 84.2 | 88.0 | 117.3 | 134.0 |
| 206 | 164.5 | 80.6 | 88.2 | 84.7 | 88.5 | 117.9 | 134.8 |
| 207 | 165.3 | 81.0 | 88.7 | 85.1 | 89.0 | 118.5 | 135.4 |
| 208 | 166.1 | 81.5 | 89.2 | 85.6 | 89.5 | 119.1 | 136.1 |
| 209 | 166.9 | 82.0 | 89.7 | 86.1 | 90.0 | 119.7 | 136.8 |
| 210 | 167.7 | 82.4 | 90.1 | 86.5 | 90.5 | 120.4 | 137.5 |
| 211 | 168.5 | 82.8 | 90.6 | 87.0 | 91.0 | 121.0 | 138.3 |
| 212 | 169.3 | 83.3 | 91.1 | 87.5 | 91.5 | 121.6 | 138.9 |
| 213 | 170.1 | 83.8 | 91.6 | 88.0 | 92.0 | 122.3 | 139.7 |
| 214 | 170.9 | 84.2 | 92.1 | 88.4 | 92.5 | 122.9 | 140.3 |
| 215 | 171.7 | 84.6 | 92.5 | 88.8 | 92.9 | 123.6 | 141.1 |
| 216 | 172.5 | 85.1 | 93.0 | 89.3 | 93.4 | 124.2 | 141.9 |
| 217 | 173.3 | 85.6 | 93.5 | 89.8 | 93.9 | 124.8 | 142.5 |
| 218 | 174.1 | 86.1 | 94.0 | 90.3 | 94.4 | 125.5 | 143.3 |
| 219 | 174.9 | 86.5 | 94.5 | 90.8 | 94.9 | 126.2 | 144.0 |
| 220 | 175.7 | 86.9 | 94.9 | 91.2 | 95.3 | 126.9 | 144.7 |
| 221 | 176.5 | 87.4 | 95.5 | 91.7 | 95.8 | 127.5 | 145.5 |
| 222 | 177.3 | 87.9 | 96.0 | 92.2 | 96.4 | 128.1 | 146.1 |
| 223 | 178.1 | 88.4 | 96.5 | 92.7 | 96.9 | 128.8 | 146.9 |
| 224 | 178.9 | 88.8 | 97.0 | 93.2 | 97.4 | 129.4 | 147.6 |
| 225 | 179.7 | 89.2 | 97.4 | 93.6 | 97.8 | 130.1 | 148.3 |
| 226 | 180.5 | 89.7 | 97.9 | 94.1 | 98.3 | 130.7 | 149.1 |
| 227 | 181.3 | 90.2 | 98.5 | 94.6 | 98.8 | 131.3 | 149.7 |
| 228 | 182.1 | 90.7 | 99.0 | 95.1 | 99.4 | 132.0 | 150.5 |
| 229 | 182.9 | 91.2 | 99.5 | 95.6 | 99.9 | 132.6 | 151.2 |

TABLE 16. (*Continued.*)
30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 230 | 183.7 | 91.6 | 99.9 | 96.0 | 100.3 | 133.3 | 151.9 |
| 231 | 184.5 | 92.1 | 100.4 | 96.5 | 100.8 | 133.9 | 152.7 |
| 232 | 185.3 | 92.6 | 101.0 | 97.1 | 101.3 | 134.6 | 153.3 |
| 233 | 186.1 | 93.1 | 101.5 | 97.6 | 101.9 | 135.3 | 154.1 |
| 234 | 186.9 | 93.5 | 102.0 | 98.1 | 102.4 | 135.9 | 154.8 |
| 235 | 187.7 | 93.9 | 102.5 | 98.5 | 102.8 | 136.6 | 155.5 |
| 236 | 188.5 | 94.5 | 103.0 | 99.0 | 103.3 | 137.2 | 156.3 |
| 237 | 189.3 | 94.9 | 103.5 | 99.5 | 103.8 | 137.8 | 156.9 |
| 238 | 190.1 | 95.4 | 104.0 | 100.0 | 104.4 | 138.5 | 157.7 |
| 239 | 190.9 | 95.9 | 104.5 | 100.5 | 104.9 | 139.1 | 158.5 |
| 240 | 191.7 | 96.3 | 105.0 | 100.9 | 105.3 | 139.8 | 159.2 |
| 241 | 192.5 | 96.8 | 105.5 | 101.4 | 105.8 | 140.5 | 160.0 |
| 242 | 193.3 | 97.3 | 106.0 | 101.9 | 106.4 | 141.1 | 160.6 |
| 243 | 194.1 | 97.8 | 106.5 | 102.4 | 106.9 | 141.8 | 161.4 |
| 244 | 194.9 | 98.3 | 107.1 | 103.0 | 107.4 | 142.5 | 162.1 |
| 245 | 195.7 | 98.7 | 107.5 | 103.4 | 107.9 | 143.2 | 162.8 |
| 246 | 196.5 | 99.2 | 107.9 | 103.9 | 108.4 | 143.8 | 163.6 |
| 247 | 197.3 | 99.7 | 108.5 | 104.4 | 108.9 | 144.4 | 164.2 |
| 248 | 198.1 | 100.2 | 109.0 | 104.9 | 109.5 | 145.1 | 165.1 |
| 249 | 198.9 | 100.7 | 109.6 | 105.4 | 110.0 | 145.8 | 165.8 |
| 250 | 199.7 | 101.1 | 110.0 | 105.8 | 110.5 | 146.5 | 166.5 |
| 251 | 200.5 | 101.7 | 110.5 | 106.3 | 110.9 | 147.1 | 167.3 |
| 252 | 201.3 | 102.2 | 111.0 | 106.9 | 111.5 | 147.7 | 167.9 |
| 253 | 202.1 | 102.7 | 111.6 | 107.4 | 112.0 | 148.5 | 168.8 |
| 254 | 202.9 | 103.2 | 112.1 | 107.9 | 112.6 | 149.1 | 169.5 |
| 255 | 203.6 | 103.6 | 112.5 | 108.3 | 113.0 | 149.7 | 170.1 |
| 256 | 204.4 | 104.0 | 113.0 | 108.8 | 113.5 | 150.4 | 170.9 |
| 257 | 205.2 | 104.5 | 113.5 | 109.3 | 114.0 | 151.1 | 171.7 |
| 258 | 206.0 | 105.0 | 114.1 | 109.8 | 114.5 | 151.7 | 172.4 |
| 259 | 206.8 | 105.6 | 114.6 | 110.4 | 115.1 | 152.3 | 173.1 |
| 260 | 207.6 | 106.1 | 115.1 | 110.9 | 115.6 | 153.0 | 173.8 |
| 261 | 208.4 | 106.5 | 115.6 | 111.3 | 116.1 | 153.7 | 174.6 |
| 262 | 209.2 | 107.0 | 116.1 | 111.8 | 116.6 | 154.4 | 175.4 |
| 263 | 210.0 | 107.5 | 116.7 | 112.4 | 117.1 | 155.0 | 176.1 |
| 264 | 210.8 | 108.1 | 117.2 | 112.9 | 117.7 | 155.7 | 176.8 |
| 265 | 211.6 | 108.6 | 117.7 | 113.4 | 118.2 | 156.4 | 177.5 |
| 266 | 212.4 | 109.0 | 118.2 | 113.9 | 118.8 | 157.1 | 178.4 |
| 267 | 213.2 | 109.5 | 118.7 | 114.4 | 119.2 | 157.8 | 179.1 |
| 268 | 214.0 | 110.1 | 119.2 | 114.9 | 119.8 | 158.4 | 179.8 |
| 269 | 214.8 | 110.6 | 119.8 | 115.5 | 120.3 | 159.1 | 180.6 |
| 270 | 215.6 | 111.1 | 120.3 | 116.0 | 120.8 | 159.8 | 181.3 |
| 271 | 216.4 | 111.5 | 120.7 | 116.4 | 121.4 | 160.5 | 182.1 |
| 272 | 217.2 | 112.1 | 121.3 | 117.0 | 121.9 | 161.2 | 182.9 |
| 273 | 218.0 | 112.6 | 121.9 | 117.5 | 122.4 | 161.8 | 183.6 |
| 274 | 218.8 | 113.2 | 122.4 | 118.1 | 123.0 | 162.5 | 184.4 |
| 275 | 219.6 | 113.7 | 122.9 | 118.6 | 123.5 | 163.2 | 185.1 |
| 276 | 220.4 | 114.1 | 123.4 | 119.0 | 124.1 | 163.9 | 185.9 |
| 277 | 221.2 | 114.6 | 124.0 | 119.6 | 124.5 | 164.6 | 186.7 |
| 278 | 222.0 | 115.2 | 124.6 | 120.2 | 125.1 | 165.2 | 187.4 |
| 279 | 222.8 | 115.7 | 125.1 | 120.7 | 125.7 | 165.9 | 188.2 |

TABLE 16. (*Continued.*)

30 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 280 | 223.6 | 116.2 | 125.6 | 121.2 | 126.2 | 166.6 | 188.9 |
| 281 | 224.4 | 116.7 | 126.1 | 121.7 | 126.8 | 167.4 | 189.7 |
| 282 | 225.2 | 117.2 | 126.7 | 122.2 | 127.3 | 168.1 | 190.5 |
| 283 | 226.0 | 117.8 | 127.2 | 122.8 | 127.8 | 168.7 | 191.2 |
| 284 | 226.8 | 118.3 | 127.8 | 123.3 | 128.4 | 169.4 | 192.0 |
| 285 | 227.6 | 118.8 | 128.3 | 123.8 | 128.9 | 170.1 | 192.7 |
| 286 | 228.4 | 119.3 | 128.8 | 124.3 | 129.5 | 170.9 | 193.5 |
| 287 | 229.2 | 119.8 | 129.4 | 124.9 | 130.0 | 171.5 | 194.3 |
| 288 | 230.0 | 120.4 | 130.0 | 125.5 | 130.5 | 172.2 | 195.1 |
| 289 | 230.8 | 121.0 | 130.5 | 126.0 | 131.1 | 172.9 | 195.8 |
| 290 | 231.6 | 121.5 | 131.0 | 126.5 | 131.6 | 173.6 | 196.5 |
| 291 | 232.4 | 122.0 | 131.5 | 127.0 | 132.2 | 174.4 | 197.4 |
| 292 | 233.2 | 122.5 | 132.1 | 127.6 | 132.7 | 175.0 | 198.1 |
| 293 | 234.0 | 123.1 | 132.7 | 128.2 | 133.3 | 175.7 | 198.9 |
| 294 | 234.8 | 123.7 | 133.3 | 128.8 | 133.9 | 176.4 | 199.7 |
| 295 | 235.6 | 124.2 | 133.8 | 129.3 | 134.4 | 177.1 | 200.4 |
| 296 | 236.4 | 124.6 | 134.3 | 129.7 | 135.0 | 177.9 | 201.2 |
| 297 | 237.2 | 125.2 | 134.9 | 130.3 | 135.5 | 178.6 | 202.0 |
| 298 | 238.0 | 125.8 | 135.5 | 130.9 | 136.1 | 179.2 | 202.7 |
| 299 | 238.8 | 126.4 | 136.0 | 131.5 | 136.7 | 179.9 | 203.5 |
| 300 | 239.6 | 126.9 | 136.5 | 132.0 | 137.2 | 180.6 | 204.2 |
| 301 | 240.4 | 127.3 | 137.0 | 132.4 | 137.8 | 181.4 | 205.1 |
| 302 | 241.2 | 127.9 | 137.6 | 133.0 | 138.3 | 182.1 | 205.8 |
| 303 | 242.0 | 128.5 | 138.2 | 133.6 | 138.9 | 182.8 | 206.6 |
| 304 | 242.8 | 129.1 | 138.8 | 134.2 | 139.5 | 183.5 | 207.4 |
| 305 | 243.6 | 129.6 | 139.3 | 134.7 | 140.0 | 184.2 | 208.1 |
| 306 | 244.4 | 130.1 | 139.8 | 135.2 | 140.6 | 185.0 | 208.9 |
| 307 | 245.2 | 130.7 | 140.4 | 135.8 | 141.1 | 185.7 | 209.7 |
| 308 | 246.0 | 131.3 | 141.0 | 136.4 | 141.7 | 186.3 | 210.5 |
| 309 | 246.8 | 131.9 | 141.6 | 137.0 | 142.3 | 187.0 | 211.3 |
| 310 | 247.6 | 132.4 | 142.1 | 137.5 | 142.8 | 187.7 | 212.0 |
| 311 | 248.4 | 132.9 | 142.6 | 138.0 | 143.4 | 188.5 | 212.8 |
| 312 | 249.2 | 133.5 | 143.2 | 138.6 | 143.9 | 189.2 | 213.6 |
| 313 | 250.0 | 134.1 | 143.8 | 139.2 | 144.5 | 189.9 | 214.4 |
| 314 | 250.8 | 134.7 | 144.4 | 139.8 | 145.1 | 190.6 | 215.2 |
| 315 | 251.6 | 135.2 | 144.9 | 140.3 | 145.6 | 191.3 | 215.9 |
| 316 | 252.4 | 135.7 | 145.4 | 140.8 | 146.3 | 192.1 | 216.8 |
| 317 | 253.2 | 136.3 | 146.1 | 141.5 | 146.8 | 192.8 | 217.6 |
| 318 | 254.0 | 136.9 | 146.7 | 142.1 | 147.4 | 193.5 | 218.3 |
| 319 | 254.8 | 137.5 | 147.3 | 142.7 | 148.0 | 194.3 | 219.1 |
| 320 | 255.6 | 138.0 | 147.8 | 143.2 | 148.5 | 195.0 | 219.8 |
| 321 | 256.4 | 138.5 | 148.3 | 143.7 | 149.2 | 195.8 | 220.7 |
| 322 | 257.2 | 139.2 | 148.9 | 144.3 | 149.7 | 196.5 | 221.5 |
| 323 | 258.0 | 139.8 | 149.5 | 144.9 | 150.3 | 197.2 | 222.3 |
| 324 | 258.8 | 140.4 | 150.1 | 145.5 | 150.9 | 197.9 | 223.1 |
| 325 | 259.6 | 140.9 | 150.6 | 146.0 | 151.4 | 198.6 | 223.8 |
| 326 | 260.4 | 141.4 | 151.1 | 146.5 | 152.1 | 199.4 | 224.7 |
| 327 | 261.2 | 142.1 | 151.7 | 147.2 | 152.6 | 200.1 | 225.5 |
| 328 | 262.0 | 142.7 | 152.3 | 147.8 | 153.2 | 200.8 | 226.3 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose. $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 126 | 100.6 | 44.9 | 49.4 | 47.5 | 49.8 | 71.8 | 83.4 |
| 127 | 101.4 | 45.3 | 49.8 | 47.9 | 50.2 | 72.4 | 84.0 |
| 128 | 102.2 | 45.7 | 50.3 | 48.3 | 50.6 | 73.0 | 84.6 |
| 129 | 103.0 | 46.1 | 50.7 | 48.7 | 51.0 | 73.6 | 85.4 |
| 130 | 103.8 | 46.4 | 51.1 | 49.1 | 51.4 | 74.2 | 86.0 |
| 131 | 104.6 | 46.8 | 51.5 | 49.5 | 51.8 | 74.7 | 86.7 |
| 132 | 105.4 | 47.2 | 51.9 | 49.9 | 52.2 | 75.4 | 87.4 |
| 133 | 106.2 | 47.6 | 52.3 | 50.3 | 52.6 | 75.9 | 88.0 |
| 134 | 107.0 | 48.0 | 52.7 | 50.7 | 53.1 | 76.5 | 88.8 |
| 135 | 107.8 | 48.3 | 53.1 | 51.1 | 53.5 | 77.1 | 89.4 |
| 136 | 108.6 | 48.7 | 53.5 | 51.5 | 53.9 | 77.7 | 90.1 |
| 137 | 109.4 | 49.1 | 54.0 | 51.9 | 54.3 | 78.3 | 90.8 |
| 138 | 110.2 | 49.5 | 54.4 | 52.3 | 54.7 | 78.8 | 91.4 |
| 139 | 111.0 | 49.8 | 54.8 | 52.6 | 55.1 | 79.4 | 92.2 |
| 140 | 111.8 | 50.2 | 55.2 | 53.0 | 55.6 | 80.1 | 92.8 |
| 141 | 112.6 | 50.6 | 55.6 | 53.4 | 56.0 | 80.6 | 93.5 |
| 142 | 113.4 | 51.0 | 56.0 | 53.8 | 56.4 | 81.2 | 94.2 |
| 143 | 114.2 | 51.3 | 56.4 | 54.2 | 56.8 | 81.8 | 94.8 |
| 144 | 115.0 | 51.7 | 56.8 | 54.6 | 57.2 | 82.4 | 95.6 |
| 145 | 115.8 | 52.1 | 57.3 | 55.0 | 57.6 | 83.0 | 96.2 |
| 146 | 116.6 | 52.5 | 57.7 | 55.4 | 58.0 | 83.6 | 96.9 |
| 147 | 117.4 | 52.9 | 58.1 | 55.8 | 58.5 | 84.2 | 97.6 |
| 148 | 118.2 | 53.2 | 58.5 | 56.2 | 58.9 | 84.7 | 98.2 |
| 149 | 119.0 | 53.6 | 58.9 | 56.6 | 59.3 | 85.4 | 99.0 |
| 150 | 119.8 | 54.0 | 59.3 | 57.0 | 59.7 | 86.0 | 99.6 |
| 151 | 120.6 | 54.4 | 59.7 | 57.4 | 60.1 | 86.5 | 100.3 |
| 152 | 121.4 | 54.8 | 60.1 | 57.8 | 60.5 | 87.1 | 101.0 |
| 153 | 122.2 | 55.1 | 60.6 | 58.2 | 61.0 | 87.7 | 101.7 |
| 154 | 123.0 | 55.5 | 61.0 | 58.6 | 61.4 | 88.3 | 102.4 |
| 155 | 123.8 | 55.9 | 61.4 | 59.0 | 61.8 | 89.0 | 103.1 |
| 156 | 124.6 | 56.3 | 61.8 | 59.4 | 62.2 | 89.5 | 103.8 |
| 157 | 125.4 | 56.7 | 62.2 | 59.8 | 62.6 | 90.1 | 104.4 |
| 158 | 126.2 | 57.0 | 62.6 | 60.1 | 63.0 | 90.6 | 105.1 |
| 159 | 127.0 | 57.4 | 63.1 | 60.5 | 63.5 | 91.3 | 105.8 |
| 160 | 127.8 | 57.8 | 63.5 | 60.9 | 63.9 | 91.9 | 106.5 |
| 161 | 128.6 | 58.2 | 63.9 | 61.3 | 64.3 | 92.5 | 107.2 |
| 162 | 129.4 | 58.6 | 64.3 | 61.7 | 64.7 | 93.1 | 107.9 |
| 163 | 130.2 | 58.9 | 64.7 | 62.1 | 65.1 | 93.6 | 108.5 |
| 164 | 131.0 | 59.3 | 65.2 | 62.5 | 65.6 | 94.2 | 109.3 |
| 165 | 131.8 | 59.7 | 65.6 | 62.9 | 66.0 | 94.9 | 109.9 |
| 166 | 132.6 | 60.1 | 66.0 | 63.3 | 66.4 | 95.4 | 110.6 |
| 167 | 133.4 | 60.5 | 66.4 | 63.7 | 66.8 | 96.0 | 111.3 |
| 168 | 134.2 | 60.9 | 66.8 | 64.1 | 67.3 | 96.6 | 112.0 |
| 169 | 135.0 | 61.2 | 67.2 | 64.5 | 67.7 | 97.2 | 112.6 |
| 170 | 135.8 | 61.6 | 67.7 | 64.9 | 68.1 | 97.8 | 113.4 |
| 171 | 136.6 | 62.0 | 68.1 | 65.3 | 68.5 | 98.4 | 114.1 |
| 172 | 137.4 | 62.4 | 68.5 | 65.7 | 68.9 | 99.0 | 114.7 |
| 173 | 138.2 | 62.8 | 68.9 | 66.1 | 69.4 | 99.5 | 115.4 |
| 174 | 139.0 | 63.2 | 69.3 | 66.5 | 69.8 | 100.2 | 116.2 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert. sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 175 | 139.8 | 63.6 | 69.7 | 66.9 | 70.2 | 100.8 | 116.8 |
| 176 | 140.6 | 63.9 | 70.2 | 67.3 | 70.6 | 101.4 | 117.5 |
| 177 | 141.4 | 64.3 | 70.6 | 67.7 | 71.1 | 102.0 | 118.2 |
| 178 | 142.2 | 64.7 | 71.0 | 68.1 | 71.5 | 102.5 | 118.8 |
| 179 | 143.0 | 65.1 | 71.4 | 68.5 | 71.9 | 103.2 | 119.6 |
| 180 | 143.8 | 65.5 | 71.9 | 69.0 | 72.3 | 103.8 | 120.3 |
| 181 | 144.6 | 65.9 | 72.3 | 69.4 | 72.8 | 104.4 | 121.0 |
| 182 | 145.4 | 66.3 | 72.6 | 69.8 | 73.2 | 105.0 | 121.7 |
| 183 | 146.2 | 66.7 | 73.1 | 70.2 | 73.6 | 105.5 | 122.3 |
| 184 | 147.0 | 67.1 | 73.6 | 70.6 | 74.0 | 106.2 | 123.1 |
| 185 | 147.7 | 67.4 | 74.0 | 71.0 | 74.4 | 106.7 | 123.7 |
| 186 | 148.5 | 67.8 | 74.4 | 71.4 | 74.9 | 107.3 | 124.4 |
| 187 | 149.3 | 68.2 | 74.8 | 71.8 | 75.3 | 107.9 | 125.1 |
| 188 | 150.1 | 68.6 | 75.3 | 72.2 | 75.7 | 108.5 | 125.8 |
| 189 | 150.9 | 69.0 | 75.7 | 72.6 | 76.2 | 109.1 | 126.4 |
| 190 | 151.7 | 69.4 | 76.1 | 73.0 | 76.6 | 109.7 | 127.1 |
| 191 | 152.5 | 69.8 | 76.5 | 73.4 | 77.0 | 110.3 | 127.8 |
| 192 | 153.3 | 70.2 | 77.0 | 73.8 | 77.4 | 110.9 | 128.5 |
| 193 | 154.1 | 70.6 | 77.4 | 74.3 | 77.9 | 111.5 | 129.2 |
| 194 | 154.9 | 71.0 | 77.8 | 74.7 | 78.3 | 112.1 | 129.9 |
| 195 | 155.7 | 71.4 | 78.1 | 75.1 | 78.7 | 112.7 | 130.6 |
| 196 | 156.5 | 71.8 | 78.6 | 75.5 | 79.2 | 113.3 | 131.3 |
| 197 | 157.3 | 72.1 | 79.1 | 75.9 | 79.6 | 113.9 | 132.0 |
| 198 | 158.1 | 72.5 | 79.5 | 76.3 | 80.0 | 114.5 | 132.7 |
| 199 | 158.9 | 72.9 | 79.9 | 76.7 | 80.5 | 115.1 | 133.3 |
| 200 | 159.7 | 73.3 | 80.3 | 77.1 | 80.9 | 115.7 | 134.0 |
| 201 | 160.5 | 73.7 | 80.8 | 77.5 | 81.3 | 116.3 | 134.8 |
| 202 | 161.3 | 74.1 | 81.2 | 77.9 | 81.7 | 116.8 | 135.5 |
| 203 | 162.1 | 74.5 | 81.6 | 78.3 | 82.2 | 117.5 | 136.1 |
| 204 | 162.9 | 74.9 | 82.1 | 78.8 | 82.6 | 118.1 | 136.8 |
| 205 | 163.7 | 75.3 | 82.5 | 79.2 | 83.0 | 118.7 | 137.5 |
| 206 | 164.5 | 75.7 | 82.9 | 79.6 | 83.5 | 119.3 | 138.3 |
| 207 | 165.3 | 76.1 | 83.4 | 80.0 | 83.9 | 119.9 | 139.0 |
| 208 | 166.1 | 76.5 | 83.8 | 80.4 | 84.3 | 120.5 | 139.6 |
| 209 | 166.9 | 76.9 | 84.2 | 80.8 | 84.8 | 121.1 | 140.3 |
| 210 | 167.7 | 77.3 | 84.6 | 81.2 | 85.2 | 121.7 | 141.0 |
| 211 | 168.5 | 77.7 | 85.1 | 81.7 | 85.6 | 122.3 | 141.7 |
| 212 | 169.3 | 78.1 | 85.5 | 82.1 | 86.1 | 122.9 | 142.4 |
| 213 | 170.1 | 78.5 | 86.0 | 82.5 | 86.5 | 123.5 | 143.1 |
| 214 | 170.9 | 78.9 | 86.4 | 82.9 | 87.0 | 124.1 | 143.8 |
| 215 | 171.7 | 79.3 | 86.8 | 83.3 | 87.4 | 124.7 | 144.5 |
| 216 | 172.5 | 79.7 | 87.2 | 83.7 | 87.8 | 125.3 | 145.2 |
| 217 | 173.3 | 80.1 | 87.7 | 84.2 | 88.2 | 125.9 | 145.9 |
| 218 | 174.1 | 80.5 | 88.1 | 84.6 | 88.7 | 126.5 | 146.6 |
| 219 | 174.9 | 80.9 | 88.6 | 85.0 | 89.1 | 127.1 | 147.3 |
| 220 | 175.7 | 81.3 | 89.0 | 85.4 | 89.5 | 127.7 | 148.0 |
| 221 | 176.5 | 81.7 | 89.4 | 85.8 | 90.0 | 128.3 | 148.8 |
| 222 | 177.3 | 82.1 | 89.8 | 86.4 | 90.4 | 128.9 | 149.5 |
| 223 | 178.1 | 82.5 | 90.3 | 86.7 | 90.9 | 129.5 | 150.1 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 224 | 178.9 | 82.9 | 90.7 | 87.1 | 91.3 | 130.1 | 150.8 |
| 225 | 179.7 | 83.3 | 91.1 | 87.5 | 91.7 | 130.7 | 151.5 |
| 226 | 180.5 | 83.7 | 91.6 | 87.9 | 92.2 | 131.3 | 152.3 |
| 227 | 181.3 | 84.1 | 92.0 | 88.3 | 92.6 | 131.9 | 153.0 |
| 228 | 182.1 | 84.5 | 92.5 | 88.8 | 93.1 | 132.5 | 153.6 |
| 229 | 182.9 | 84.9 | 92.9 | 89.2 | 93.5 | 133.1 | 154.3 |
| 230 | 183.7 | 85.3 | 93.3 | 89.6 | 93.9 | 133.7 | 155.0 |
| 231 | 184.5 | 85.7 | 93.8 | 90.0 | 94.4 | 134.3 | 155.8 |
| 232 | 185.3 | 86.1 | 94.2 | 90.4 | 94.8 | 134.9 | 156.5 |
| 233 | 186.1 | 86.5 | 94.7 | 90.9 | 95.3 | 135.5 | 157.2 |
| 234 | 186.9 | 86.9 | 95.1 | 91.3 | 95.7 | 136.1 | 157.8 |
| 235 | 187.7 | 87.3 | 95.5 | 91.7 | 96.1 | 136.7 | 158.5 |
| 236 | 188.5 | 87.7 | 96.0 | 92.1 | 96.6 | 137.3 | 159.3 |
| 237 | 189.3 | 88.1 | 96.4 | 92.6 | 97.0 | 137.9 | 160.0 |
| 238 | 190.1 | 88.5 | 96.9 | 93.0 | 97.5 | 138.6 | 160.7 |
| 239 | 190.9 | 88.9 | 97.3 | 93.4 | 97.9 | 139.2 | 161.4 |
| 240 | 191.7 | 89.3 | 97.7 | 93.8 | 98.3 | 139.8 | 162.1 |
| 241 | 192.5 | 89.6 | 98.1 | 94.2 | 98.7 | 140.4 | 162.8 |
| 242 | 193.3 | 90.2 | 98.6 | 94.7 | 99.2 | 141.0 | 163.5 |
| 243 | 194.1 | 90.6 | 99.1 | 95.1 | 99.7 | 141.6 | 164.2 |
| 244 | 194.9 | 91.0 | 99.5 | 95.5 | 100.2 | 142.2 | 164.9 |
| 245 | 195.7 | 91.4 | 99.9 | 95.9 | 100.6 | 142.8 | 165.6 |
| 246 | 196.5 | 91.8 | 100.4 | 96.4 | 101.1 | 143.4 | 166.3 |
| 247 | 197.3 | 92.2 | 100.8 | 96.8 | 101.5 | 144.0 | 167.0 |
| 248 | 198.1 | 92.6 | 101.3 | 97.2 | 101.9 | 144.6 | 167.7 |
| 249 | 198.9 | 93.0 | 101.7 | 97.6 | 102.2 | 145.4 | 168.4 |
| 250 | 199.7 | 93.4 | 102.1 | 98.0 | 102.6 | 145.9 | 169.1 |
| 251 | 200.5 | 93.8 | 102.6 | 98.5 | 103.2 | 146.5 | 169.8 |
| 252 | 201.3 | 94.3 | 103.0 | 98.9 | 103.7 | 147.1 | 170.6 |
| 253 | 202.1 | 94.7 | 103.5 | 99.4 | 104.2 | 147.7 | 171.3 |
| 254 | 202.9 | 95.1 | 103.9 | 99.8 | 104.6 | 148.3 | 172.0 |
| 255 | 203.6 | 95.4 | 104.3 | 100.1 | 105.0 | 148.9 | 172.6 |
| 256 | 204.4 | 95.8 | 104.7 | 100.5 | 105.4 | 149.5 | 173.3 |
| 257 | 205.2 | 96.2 | 105.1 | 100.9 | 105.8 | 150.1 | 174.0 |
| 258 | 206.0 | 96.6 | 105.6 | 101.4 | 106.3 | 150.7 | 174.7 |
| 259 | 206.8 | 97.1 | 106.1 | 101.9 | 106.8 | 151.3 | 175.4 |
| 260 | 207.6 | 97.5 | 106.5 | 102.3 | 107.2 | 152.0 | 176.1 |
| 261 | 208.4 | 97.9 | 106.9 | 102.7 | 107.6 | 152.6 | 176.9 |
| 262 | 209.2 | 98.3 | 107.4 | 103.1 | 108.1 | 153.2 | 177.6 |
| 263 | 210.0 | 98.7 | 107.9 | 103.6 | 108.5 | 153.8 | 178.3 |
| 264 | 210.8 | 99.1 | 108.3 | 104.0 | 109.0 | 154.4 | 179.0 |
| 265 | 211.6 | 99.5 | 108.7 | 104.4 | 109.4 | 155.0 | 179.7 |
| 266 | 212.4 | 99.9 | 109.2 | 104.8 | 109.9 | 155.6 | 180.4 |
| 267 | 213.2 | 100.4 | 109.6 | 105.3 | 110.3 | 156.3 | 181.2 |
| 268 | 214.0 | 100.8 | 110.1 | 105.7 | 110.8 | 156.9 | 181.9 |
| 269 | 214.8 | 101.2 | 110.6 | 106.2 | 111.3 | 157.5 | 182.6 |
| 270 | 215.6 | 101.6 | 111.0 | 106.6 | 111.7 | 158.1 | 183.3 |
| 271 | 216.4 | 102.0 | 111.4 | 107.0 | 112.1 | 158.7 | 184.0 |
| 272 | 217.2 | 102.5 | 111.9 | 107.5 | 112.6 | 159.4 | 184.7 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 273 | 218.0 | 102.9 | 112.3 | 107.9 | 113.1 | 160.0 | 185.4 |
| 274 | 218.8 | 103.3 | 112.8 | 108.3 | 113.5 | 160.5 | 186.1 |
| 275 | 219.6 | 103.7 | 113.2 | 108.7 | 113.9 | 161.2 | 186.8 |
| 276 | 220.4 | 104.1 | 113.7 | 109.2 | 114.4 | 161.8 | 187.6 |
| 277 | 221.2 | 104.5 | 114.1 | 109.6 | 114.9 | 162.5 | 188.3 |
| 278 | 222.0 | 105.0 | 114.6 | 110.1 | 115.3 | 163.1 | 189.0 |
| 279 | 222.8 | 105.4 | 115.1 | 110.5 | 115.8 | 163.6 | 189.7 |
| 280 | 223.6 | 105.8 | 115.5 | 110.9 | 116.2 | 164.3 | 190.4 |
| 281 | 224.4 | 106.2 | 115.9 | 111.3 | 116.7 | 164.9 | 191.2 |
| 282 | 225.2 | 106.7 | 116.4 | 111.8 | 117.1 | 165.6 | 191.9 |
| 283 | 226.0 | 107.1 | 116.9 | 112.3 | 117.6 | 166.2 | 192.6 |
| 284 | 226.8 | 107.5 | 117.4 | 112.7 | 118.1 | 166.8 | 193.3 |
| 285 | 227.6 | 107.9 | 117.8 | 113.1 | 118.5 | 167.4 | 194.0 |
| 286 | 228.4 | 108.3 | 118.2 | 113.5 | 118.9 | 168.0 | 194.8 |
| 287 | 229.2 | 108.8 | 118.7 | 114.0 | 119.4 | 168.7 | 195.5 |
| 288 | 230.0 | 109.2 | 119.2 | 114.5 | 119.9 | 169.3 | 196.2 |
| 289 | 230.8 | 109.6 | 119.6 | 114.9 | 120.4 | 169.9 | 196.9 |
| 290 | 231.6 | 110.1 | 120.1 | 115.4 | 120.8 | 170.6 | 197.6 |
| 291 | 232.4 | 110.5 | 120.5 | 115.8 | 121.2 | 171.2 | 198.4 |
| 292 | 233.2 | 110.9 | 121.0 | 116.2 | 121.7 | 171.8 | 199.1 |
| 293 | 234.0 | 111.3 | 121.4 | 116.6 | 122.2 | 172.4 | 199.8 |
| 294 | 234.8 | 111.8 | 121.9 | 117.1 | 122.7 | 173.0 | 200.5 |
| 295 | 235.6 | 112.2 | 122.4 | 117.6 | 123.1 | 173.7 | 201.2 |
| 296 | 236.4 | 112.6 | 122.8 | 118.0 | 123.5 | 174.3 | 202.0 |
| 297 | 237.2 | 113.0 | 123.3 | 118.4 | 124.0 | 174.9 | 202.7 |
| 298 | 238.0 | 113.5 | 123.7 | 118.9 | 124.5 | 175.5 | 203.4 |
| 299 | 238.8 | 113.9 | 124.2 | 119.3 | 125.0 | 176.1 | 204.1 |
| 300 | 239.6 | 114.3 | 124.7 | 119.8 | 125.5 | 176.8 | 204.8 |
| 301 | 240.4 | 114.7 | 125.1 | 120.2 | 125.9 | 177.4 | 205.6 |
| 302 | 241.2 | 115.2 | 125.5 | 120.6 | 126.3 | 178.1 | 206.3 |
| 303 | 242.0 | 115.6 | 126.0 | 121.1 | 126.8 | 178.7 | 207.0 |
| 304 | 242.8 | 116.1 | 126.5 | 121.6 | 127.3 | 179.2 | 207.7 |
| 305 | 243.6 | 116.5 | 127.0 | 122.0 | 127.8 | 179.9 | 208.4 |
| 306 | 244.4 | 116.9 | 127.4 | 122.4 | 128.2 | 180.5 | 209.2 |
| 307 | 245.2 | 117.3 | 127.9 | 122.9 | 128.7 | 181.2 | 209.9 |
| 308 | 246.0 | 117.8 | 128.3 | 123.3 | 129.1 | 181.8 | 210.6 |
| 309 | 246.8 | 118.2 | 128.8 | 123.8 | 129.6 | 182.4 | 211.3 |
| 310 | 247.6 | 118.6 | 129.2 | 124.2 | 130.0 | 183.0 | 212.0 |
| 311 | 248.4 | 119.1 | 129.7 | 124.7 | 130.5 | 183.6 | 212.8 |
| 312 | 249.2 | 119.5 | 130.2 | 125.1 | 131.0 | 184.3 | 213.6 |
| 313 | 250.0 | 119.9 | 130.7 | 125.6 | 131.5 | 184.9 | 214.3 |
| 314 | 250.8 | 120.4 | 131.2 | 126.1 | 132.0 | 185.5 | 215.0 |
| 315 | 251.6 | 120.8 | 131.6 | 126.5 | 132.4 | 186.2 | 215.7 |
| 316 | 252.4 | 121.2 | 132.0 | 126.9 | 132.9 | 186.8 | 216.5 |
| 317 | 253.2 | 121.7 | 132.5 | 127.4 | 133.3 | 187.4 | 217.2 |
| 318 | 254.0 | 122.1 | 133.0 | 127.8 | 133.8 | 188.0 | 217.9 |
| 319 | 254.8 | 122.6 | 133.5 | 128.3 | 134.3 | 188.6 | 218.6 |
| 320 | 255.6 | 123.0 | 133.9 | 128.7 | 134.7 | 189.3 | 219.3 |
| 321 | 256.4 | 123.4 | 134.4 | 129.2 | 135.2 | 189.9 | 220.1 |
| 322 | 257.2 | 123.9 | 134.8 | 129.6 | 135.7 | 190.6 | 220.8 |

TABLE 16. (*Continued.*)

50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 323 | 258.0 | 124.3 | 135.3 | 130.1 | 136.2 | 191.2 | 221.5 |
| 324 | 258.8 | 124.8 | 135.8 | 130.6 | 136.7 | 191.7 | 222.2 |
| 325 | 259.6 | 125.2 | 136.2 | 131.0 | 137.1 | 192.4 | 222.9 |
| 326 | 260.4 | 125.6 | 136.7 | 131.4 | 137.6 | 193.0 | 223.7 |
| 327 | 261.2 | 126.1 | 137.2 | 131.9 | 138.1 | 193.7 | 224.5 |
| 328 | 262.0 | 126.5 | 137.7 | 132.4 | 138.6 | 194.3 | 225.2 |
| 329 | 262.7 | 126.9 | 138.1 | 132.8 | 139.0 | 194.9 | 225.8 |
| 330 | 263.5 | 127.4 | 138.6 | 133.3 | 139.4 | 195.5 | 226.6 |
| 331 | 264.3 | 127.8 | 139.0 | 133.7 | 139.9 | 196.1 | 227.3 |
| 332 | 265.1 | 128.3 | 139.5 | 134.2 | 140.4 | 196.8 | 228.0 |
| 333 | 265.9 | 128.7 | 140.0 | 134.6 | 140.9 | 197.3 | 228.7 |
| 334 | 266.7 | 129.1 | 140.4 | 135.0 | 141.3 | 198.0 | 229.4 |
| 335 | 267.5 | 129.6 | 140.9 | 135.5 | 141.8 | 198.6 | 230.6 |
| 336 | 268.3 | 130.1 | 141.4 | 136.0 | 142.3 | 199.2 | 231.0 |
| 337 | 269.1 | 130.5 | 141.9 | 136.5 | 142.8 | 199.9 | 231.7 |
| 338 | 269.9 | 131.0 | 142.4 | 137.0 | 143.3 | 200.5 | 232.4 |
| 339 | 270.7 | 131.4 | 142.8 | 137.4 | 143.7 | 201.1 | 233.1 |
| 340 | 271.5 | 131.8 | 143.3 | 137.8 | 144.2 | 201.8 | 233.9 |
| 341 | 272.3 | 132.3 | 143.8 | 138.3 | 144.7 | 202.4 | 234.6 |
| 342 | 273.1 | 132.7 | 144.3 | 138.8 | 145.2 | 203.1 | 235.3 |
| 343 | 273.9 | 133.2 | 144.8 | 139.3 | 145.7 | 203.7 | 236.1 |
| 344 | 274.7 | 133.6 | 145.2 | 139.7 | 146.1 | 204.3 | 236.8 |
| 345 | 275.5 | 134.1 | 145.7 | 140.2 | 146.6 | 205.0 | 237.6 |
| 346 | 276.3 | 134.5 | 146.2 | 140.6 | 147.1 | 205.6 | 238.3 |
| 347 | 277.1 | 135.0 | 146.7 | 141.1 | 147.6 | 206.3 | 239.0 |
| 348 | 277.9 | 135.5 | 147.1 | 141.6 | 148.1 | 206.9 | 239.7 |
| 349 | 278.7 | 135.9 | 147.5 | 142.0 | 148.5 | 207.6 | 240.4 |
| 350 | 279.5 | 136.3 | 148.0 | 142.4 | 149.0 | 208.2 | 241.3 |
| 351 | 280.3 | 136.8 | 148.5 | 142.9 | 149.5 | 208.8 | 242.0 |
| 352 | 281.1 | 137.3 | 149.0 | 143.4 | 150.0 | 209.5 | 242.7 |
| 353 | 281.9 | 137.7 | 149.5 | 143.9 | 150.5 | 210.1 | 243.4 |
| 354 | 282.7 | 138.1 | 149.9 | 144.3 | 150.9 | 210.8 | 244.1 |
| 355 | 283.5 | 138.6 | 150.4 | 144.8 | 151.4 | 211.4 | 245.0 |
| 356 | 284.3 | 139.1 | 150.9 | 145.3 | 151.9 | 212.0 | 245.7 |
| 357 | 285.1 | 139.5 | 151.4 | 145.7 | 152.5 | 212.7 | 246.4 |
| 358 | 285.9 | 140.0 | 151.9 | 146.2 | 153.0 | 213.3 | 247.1 |
| 359 | 286.7 | 140.4 | 152.3 | 146.6 | 153.4 | 214.0 | 247.8 |
| 360 | 287.5 | 140.9 | 152.8 | 147.1 | 153.9 | 214.6 | 248.7 |
| 361 | 288.3 | 141.3 | 153.3 | 147.6 | 154.4 | 215.2 | 249.4 |
| 362 | 289.1 | 141.8 | 153.8 | 148.1 | 154.9 | 215.9 | 250.1 |
| 363 | 289.9 | 142.3 | 154.3 | 148.6 | 155.4 | 216.5 | 250.9 |
| 364 | 290.7 | 142.7 | 154.7 | 149.0 | 155.8 | 217.2 | 251.6 |
| 365 | 291.5 | 143.2 | 155.2 | 149.5 | 156.3 | 217.8 | 252.4 |
| 366 | 292.3 | 143.6 | 155.8 | 150.0 | 156.8 | 218.4 | 253.1 |
| 367 | 293.1 | 144.1 | 156.3 | 150.5 | 157.3 | 219.1 | 253.8 |
| 368 | 293.9 | 144.6 | 156.8 | 151.0 | 157.8 | 219.7 | 254.6 |
| 369 | 294.7 | 145.0 | 157.2 | 151.4 | 158.2 | 220.4 | 255.3 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose | Lactose. $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 370 | 295.5 | 145.4 | 157.7 | 151.8 | 158.8 | 221.0 | 256.1 |
| 371 | 296.3 | 145.9 | 158.2 | 152.3 | 159.3 | 221.6 | 256.8 |
| 372 | 297.1 | 146.4 | 158.7 | 152.8 | 159.8 | 222.3 | 257.6 |
| 373 | 297.9 | 146.9 | 159.2 | 153.3 | 160.3 | 222.9 | 258.3 |
| 374 | 298.7 | 147.3 | 159.6 | 153.7 | 160.7 | 223.6 | 259.0 |
| 375 | 299.5 | 147.8 | 160.1 | 154.2 | 161.2 | 224.2 | 259.8 |
| 376 | 300.3 | 148.2 | 160.6 | 154.7 | 161.7 | 224.8 | 260.5 |
| 377 | 301.1 | 148.7 | 161.1 | 155.2 | 162.3 | 225.5 | 261.3 |
| 378 | 301.9 | 149.2 | 161.7 | 155.7 | 162.8 | 226.1 | 262.0 |
| 379 | 302.7 | 149.6 | 162.1 | 156.1 | 163.2 | 226.8 | 262.7 |
| 380 | 303.5 | 150.1 | 162.6 | 156.6 | 163.7 | 227.4 | 263.5 |
| 381 | 304.3 | 150.6 | 163.1 | 157.1 | 164.2 | 228.0 | 264.3 |
| 382 | 305.1 | 151.0 | 163.6 | 157.6 | 164.7 | 228.8 | 265.0 |
| 383 | 305.9 | 151.5 | 164.1 | 158.1 | 165.3 | 229.4 | 265.7 |
| 384 | 306.7 | 151.9 | 164.5 | 158.5 | 165.7 | 230.1 | 266.4 |
| 385 | 307.5 | 152.4 | 165.1 | 159.0 | 166.2 | 230.7 | 267.3 |
| 386 | 308.3 | 152.9 | 165.6 | 159.5 | 166.7 | 231.3 | 268.0 |
| 387 | 309.1 | 153.4 | 166.1 | 160.0 | 167.2 | 232.0 | 268.7 |
| 388 | 309.9 | 153.9 | 166.6 | 160.5 | 167.7 | 232.6 | 269.5 |
| 389 | 310.7 | 154.3 | 167.0 | 160.9 | 168.1 | 233.3 | 270.2 |
| 390 | 311.5 | 154.8 | 167.5 | 161.4 | 168.7 | 233.9 | 271.0 |
| 391 | 312.3 | 155.3 | 168.0 | 161.9 | 169.2 | 234.5 | 271.8 |
| 392 | 313.1 | 155.7 | 168.6 | 162.4 | 169.7 | 235.2 | 272.5 |
| 393 | 313.9 | 156.2 | 169.1 | 162.9 | 170.2 | 235.8 | 273.2 |
| 394 | 314.7 | 156.6 | 169.5 | 163.3 | 170.6 | 236.5 | 274.0 |
| 395 | 315.5 | 157.1 | 170.0 | 163.8 | 171.2 | 237.2 | 274.8 |
| 396 | 316.3 | 157.6 | 170.5 | 164.3 | 171.7 | 237.8 | 275.5 |
| 397 | 317.0 | 158.0 | 170.9 | 164.7 | 172.1 | 238.4 | 276.2 |
| 398 | 317.8 | 158.5 | 171.5 | 165.3 | 172.6 | 239.0 | 276.9 |
| 399 | 318.6 | 159.0 | 172.0 | 165.8 | 173.1 | 239.7 | 277.6 |
| 400 | 319.4 | 159.4 | 172.4 | 166.2 | 173.6 | 240.3 | 278.4 |
| 401 | 320.2 | 159.9 | 172.9 | 166.7 | 174.1 | 241.0 | 279.2 |
| 402 | 321.0 | 160.4 | 173.4 | 167.2 | 174.6 | 241.6 | 279.9 |
| 403 | 321.8 | 160.9 | 174.0 | 167.7 | 175.2 | 242.2 | 280.7 |
| 404 | 322.6 | 161.4 | 174.5 | 168.2 | 175.7 | 242.9 | 281.4 |
| 405 | 323.4 | 161.8 | 174.9 | 168.6 | 176.2 | 243.6 | 282.2 |
| 406 | 324.2 | 162.3 | 175.4 | 169.1 | 176.6 | 244.3 | 282.9 |
| 407 | 325.0 | 162.8 | 176.0 | 169.7 | 177.2 | 244.9 | 283.7 |
| 408 | 325.8 | 163.3 | 176.5 | 170.2 | 177.7 | 245.5 | 284.4 |
| 409 | 326.6 | 163.8 | 177.0 | 170.7 | 178.2 | 246.2 | 285.2 |
| 410 | 327.4 | 164.2 | 177.5 | 171.1 | 178.7 | 246.9 | 286.0 |
| 411 | 328.2 | 164.7 | 178.0 | 171.6 | 179.2 | 247.6 | 286.7 |
| 412 | 329.0 | 165.2 | 178.5 | 172.1 | 179.7 | 248.2 | 287.5 |
| 413 | 329.8 | 165.7 | 179.0 | 172.6 | 180.2 | 248.8 | 288.2 |
| 414 | 330.6 | 166.2 | 179.5 | 173.1 | 180.7 | 249.5 | 289.0 |
| 415 | 331.4 | 166.6 | 180.0 | 173.6 | 181.2 | 250.1 | 289.8 |
| 416 | 332.2 | 167.1 | 180.5 | 174.1 | 181.7 | 250.8 | 290.5 |
| 417 | 333.0 | 167.6 | 181.0 | 174.6 | 182.3 | 251.5 | 291.3 |
| 418 | 333.8 | 168.1 | 181.6 | 175.1 | 182.8 | 252.1 | 292.0 |
| 419 | 334.6 | 168.6 | 182.1 | 175.6 | 183.3 | 252.8 | 292.8 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert. sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 420 | 335.4 | 169.1 | 182.5 | 176.1 | 183.8 | 253.4 | 293.6 |
| 421 | 336.2 | 169.6 | 183.0 | 176.6 | 184.3 | 254.1 | 294.3 |
| 422 | 337.0 | 170.1 | 183.6 | 177.1 | 184.8 | 254.7 | 295.1 |
| 423 | 337.8 | 170.6 | 184.1 | 177.6 | 185.4 | 255.4 | 295.8 |
| 424 | 338.6 | 171.1 | 184.6 | 178.1 | 185.9 | 256.1 | 296.6 |
| 425 | 339.4 | 171.5 | 185.0 | 178.6 | 186.4 | 256.7 | 297.4 |
| 426 | 340.2 | 172.0 | 185.6 | 179.1 | 186.9 | 257.4 | 298.1 |
| 427 | 341.0 | 172.5 | 186.1 | 179.6 | 187.4 | 258.0 | 298.9 |
| 428 | 341.8 | 173.1 | 186.6 | 180.1 | 188.0 | 258.6 | 299.6 |
| 429 | 342.6 | 173.6 | 187.1 | 180.6 | 188.5 | 259.3 | 300.4 |
| 430 | 343.4 | 174.0 | 187.6 | 181.1 | 189.0 | 260.0 | 301.2 |
| 431 | 344.2 | 174.5 | 188.1 | 181.6 | 189.5 | 260.7 | 301.9 |
| 432 | 345.0 | 175.0 | 188.7 | 182.1 | 190.0 | 261.3 | 302.7 |
| 433 | 345.8 | 175.5 | 189.2 | 182.6 | 190.6 | 261.9 | 303.4 |
| 434 | 346.6 | 176.0 | 189.7 | 183.1 | 191.1 | 262.6 | 304.2 |
| 435 | 347.4 | 176.5 | 190.2 | 183.6 | 191.6 | 263.3 | 305.0 |
| 436 | 348.2 | 177.0 | 190.7 | 184.1 | 192.1 | 264.0 | 305.7 |
| 437 | 349.0 | 177.5 | 191.3 | 184.7 | 192.6 | 264.6 | 306.5 |
| 438 | 349.8 | 178.0 | 191.8 | 185.2 | 193.2 | 265.2 | 307.3 |
| 439 | 350.6 | 178.5 | 192.3 | 185.7 | 193.7 | 265.9 | 308.0 |
| 440 | 351.4 | 179.0 | 192.8 | 186.2 | 194.2 | 266.6 | 308.8 |
| 441 | 352.2 | 179.5 | 193.3 | 186.7 | 194.7 | 267.3 | 309.5 |
| 442 | 353.0 | 180.0 | 193.8 | 187.2 | 195.2 | 267.9 | 310.3 |
| 443 | 353.8 | 180.5 | 194.4 | 187.7 | 195.8 | 268.5 | 311.1 |
| 444 | 354.6 | 181.0 | 194.9 | 188.2 | 196.3 | 269.2 | 311.8 |
| 445 | 355.4 | 181.5 | 195.4 | 188.7 | 196.8 | 269.9 | 312.6 |
| 446 | 356.2 | 182.0 | 195.9 | 189 2 | 197.3 | 270.6 | 313.5 |
| 447 | 357.0 | 182.5 | 196.4 | 189.7 | 197.9 | 271.2 | 314.2 |
| 448 | 357.8 | 183.1 | 197.0 | 190.3 | 198.4 | 271.8 | 315.0 |
| 449 | 358.6 | 183.6 | 197.5 | 190.8 | 198.9 | 272.5 | 315.7 |
| 450 | 359.4 | 184.0 | 198.0 | 191.3 | 199.4 | 273.2 | 316.5 |
| 451 | 360.2 | 184.5 | 198.5 | 191.8 | 199.9 | 273.9 | 317.2 |
| 452 | 361.0 | 185.1 | 199.0 | 192.3 | 200.5 | 274.5 | 318.0 |
| 453 | 361.8 | 185.6 | 199.6 | 192.9 | 201.1 | 275.2 | 318.8 |
| 454 | 362.6 | 186.1 | 200.1 | 193.4 | 201.6 | 275.9 | 319.6 |
| 455 | 363.4 | 186.6 | 200.7 | 193.9 | 202.1 | 276.6 | 320.3 |
| 456 | 364.2 | 187.1 | 201.1 | 194.4 | 202.6 | 277.3 | 321.1 |
| 457 | 365.0 | 187.6 | 201.7 | 194.9 | 203.3 | 277.9 | 321.9 |
| 458 | 365.8 | 188.2 | 202.3 | 195.5 | 203.7 | 278.5 | 322.6 |
| 459 | 366.6 | 188.7 | 202.8 | 196.0 | 204.2 | 279.2 | 323.4 |
| 460 | 367.4 | 189.1 | 203.3 | 196.5 | 204.8 | 279.9 | 324.2 |
| 461 | 368.2 | 189.6 | 203.8 | 197.0 | 205.3 | 280.6 | 325.0 |
| 462 | 369.0 | 190.2 | 204.3 | 197.5 | 205.8 | 281.3 | 325.7 |
| 463 | 369.8 | 190.7 | 204.9 | 198.1 | 206.4 | 281.9 | 326.5 |
| 464 | 370.6 | 191.2 | 205.4 | 198.6 | 206.9 | 282.6 | 327.3 |
| 465 | 371.4 | 191.7 | 206.0 | 199.2 | 207.5 | 283.3 | 328.1 |
| 466 | 372.2 | 192.2 | 206.4 | 199.6 | 208.0 | 284.0 | 328.8 |
| 467 | 373.0 | 192.8 | 207.0 | 200.2 | 208.5 | 284.6 | 329.6 |
| 468 | 373.7 | 193.2 | 207.5 | 200.6 | 209.0 | 285.2 | 330.3 |
| 469 | 374.5 | 193.8 | 208.1 | 201.2 | 209.6 | 285.9 | 331.1 |

TABLE 16. (*Continued.*)
50 c.c. Fehling's Solution.

| Cupric oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 470 | 375.3 | 194.3 | 208.5 | 201.7 | 210.1 | 286.5 | 331.8 |
| 471 | 376.1 | 194.8 | 209.1 | 202.2 | 210.7 | 287.2 | 332.6 |
| 472 | 376.9 | 195.3 | 209.6 | 202.7 | 211.1 | 287.9 | 333.4 |
| 473 | 377.7 | 195.8 | 210.2 | 203.2 | 211.7 | 288.6 | 334.1 |
| 474 | 378.5 | 196.4 | 210.7 | 203.8 | 212.3 | 289.2 | 334.9 |
| 475 | 379.3 | 196.9 | 211.2 | 204.3 | 212.8 | 289.9 | 335.7 |
| 476 | 380.1 | 197.4 | 211.8 | 204.9 | 213.4 | 290.6 | 336.5 |
| 477 | 380.9 | 197.9 | 212.2 | 205.3 | 213.8 | 291.3 | 337.3 |
| 478 | 381.7 | 198.5 | 212.8 | 205.9 | 214.4 | 292.0 | 338.0 |
| 479 | 382.5 | 199.0 | 213.4 | 206.5 | 215.0 | 292.7 | 338.8 |
| 480 | 383.3 | 199.5 | 213.9 | 207.0 | 215.5 | 293.3 | 339.6 |
| 481 | 384.1 | 200.1 | 214.5 | 207.6 | 216.1 | 294.0 | 340.4 |
| 482 | 384.9 | 200.5 | 215.0 | 208.0 | 216.6 | 294.7 | 341.2 |
| 483 | 385.7 | 201.1 | 215.5 | 208.6 | 217.2 | 295.4 | 342.0 |
| 484 | 386.5 | 201.7 | 216.1 | 209.2 | 217.8 | 296.1 | 342.8 |
| 485 | 387.3 | 202.2 | 216.6 | 209.7 | 218.3 | 296.7 | 343.5 |
| 486 | 388.1 | 202.7 | 217.2 | 210.2 | 218.8 | 297.4 | 344.3 |
| 487 | 388.9 | 203.2 | 217.7 | 210.7 | 219.3 | 298.1 | 345.1 |
| 488 | 389.7 | 203.8 | 218.3 | 211.3 | 219.9 | 298.8 | 345.9 |
| 489 | 390.5 | 204.3 | 218.9 | 211.9 | 220.5 | 299.5 | 346.7 |
| 490 | 391.3 | 204.8 | 219.4 | 212.4 | 221.0 | 300.1 | 347.5 |
| 491 | 392.1 | 205.4 | 219.8 | 212.9 | 221.6 | 300.8 | 348.2 |
| 492 | 392.9 | 205.9 | 220.4 | 213.4 | 222.1 | 301.5 | 349.0 |
| 493 | 393.7 | 206.5 | 221.0 | 214.0 | 222.7 | 302.2 | 349.8 |
| 494 | 394.5 | 207.0 | 221.6 | 214.6 | 223.3 | 302.9 | 350.6 |
| 495 | 395.3 | 207.5 | 222.1 | 215.1 | 223.8 | 303.5 | 351.4 |
| 496 | 396.1 | 208.1 | 222.7 | 215.7 | 224.4 | 304.2 | 352.2 |
| 497 | 396.9 | 208.6 | 223.2 | 216.2 | 224.9 | 304.9 | 352.9 |
| 498 | 397.7 | 209.2 | 223.7 | 216.7 | 225.5 | 305.6 | 353.7 |
| 499 | 398.5 | 209.7 | 224.3 | 217.3 | 226.1 | 306.3 | 354.5 |
| 500 | 399.3 | 210.2 | 224.8 | 217.8 | 226.6 | 306.9 | 355.3 |
| 501 | 400.1 | 210.8 | 225.4 | 218.4 | 227.2 | 307.6 | 356.1 |
| 502 | 400.9 | 211.3 | 225.9 | 218.9 | 227.7 | 308.3 | 356.9 |
| 503 | 401.7 | 211.9 | 226.5 | 219.5 | 228.3 | 309.0 | 357.7 |
| 504 | 402.5 | 212.5 | 227.2 | 220.1 | 228.9 | 309.7 | 358.5 |
| 505 | 403.3 | 213.0 | 227.6 | 220.6 | 229.4 | 310.3 | 359.2 |
| 506 | 404.1 | 213.6 | 228.2 | 221.2 | 230.0 | 311.0 | 360.0 |
| 507 | 404.9 | 214.0 | 228.7 | 221.6 | 230.5 | 311.7 | 360.8 |
| 508 | 405.7 | 214.6 | 229.3 | 222.2 | 231.1 | 312.4 | 361.6 |
| 509 | 406.5 | 215.2 | 229.9 | 222.8 | 231.7 | 313.1 | 362.4 |
| 510 | 407.3 | 215.7 | 230.4 | 223.3 | 232.3 | 313.7 | 363.2 |
| 511 | 408.1 | 216.3 | 231.0 | 223.9 | 232.8 | 314.4 | 364.0 |
| 512 | 408.9 | 216.8 | 231.5 | 224.4 | 233.3 | 315.1 | 364.8 |
| 513 | 409.7 | 217.4 | 232.1 | 225.0 | 233.9 | 315.8 | 365.6 |
| 514 | 410.5 | 218.0 | 232.7 | 225.6 | 234.5 | 316.5 | 366.4 |
| 515 | 411.3 | 218.5 | 233.2 | 226.1 | 235.0 | 317.1 | 367.1 |
| 516 | 412.1 | 219.1 | 233.8 | 226.7 | 235.6 | 317.8 | 367.9 |
| 517 | 412.9 | 219.6 | 234.3 | 227.2 | 236.2 | 318.5 | 368.7 |
| 518 | 413.7 | 220.2 | 234.9 | 227.8 | 236.8 | 319.2 | 369.5 |
| 519 | 414.5 | 220.8 | 235.5 | 228.4 | 237.4 | 319.9 | 370.3 |

TABLE 16. (*Concluded.*)

50 c.c. Fehling's Solution.

| Copper oxide (CuO). | Copper (Cu). | Glucose. | Fructose. | Invert sugar. | Galactose. | Lactose $C_{12}H_{22}O_{11}+H_2O$ | Maltose $C_{12}H_{22}O_{11}$ |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 520 | 415.3 | 221.3 | 236.0 | 228.9 | 237.9 | 320.6 | 371.1 |
| 521 | 416.1 | 221.9 | 236.6 | 229.5 | 238.5 | 321.3 | 371.9 |
| 522 | 416.9 | 222.4 | 237.1 | 230.0 | 239.0 | 321.9 | 372.7 |
| 523 | 417.7 | 223.0 | 237.7 | 230.6 | 239.6 | 322.6 | 373.5 |
| 524 | 418.5 | 223.6 | 238.3 | 231.2 | 240.2 | 323.3 | 374.3 |
| 525 | 419.3 | 224.2 | 238 8 | 231.7 | 240.7 | 323.9 | 375.1 |
| 526 | 420.1 | 224.7 | 239.5 | 232.4 | 241.3 | 324.7 | 375.9 |
| 527 | 420.9 | 225.2 | 240.0 | 233.0 | 241.9 | 325.4 | 376.7 |
| 528 | 421.7 | 225.8 | 240.6 | 233.5 | 242.5 | 326.1 | 377.5 |
| 529 | 422.5 | 226.4 | 241.2 | 234.1 | 243.1 | 326.8 | 378.3 |
| 530 | 423.3 | 227.0 | 241.7 | 234.6 | 243.6 | 327.4 | 379.1 |
| 531 | 424.1 | 227.6 | 242.3 | 235.2 | 244.2 | 328.1 | 379.9 |
| 532 | 424.9 | 228.1 | 242.9 | 235.8 | 244.8 | 328.9 | 380.7 |
| 533 | 425.7 | 228.7 | 243.5 | 236.4 | 245.4 | 329.6 | 381.5 |
| 534 | 426.4 | 229.2 | 244.0 | 236.9 | 245.9 | 330.2 | 382.2 |
| 535 | 427.2 | 229.7 | 244.5 | 237.4 | 246.5 | 330.9 | 383.0 |
| 536 | 428.0 | 230.3 | 245.1 | 238.0 | 247.1 | 331.6 | 383.8 |
| 537 | 428.8 | 230.9 | 245.7 | 238.6 | 247.6 | 332.2 | 384.6 |
| 538 | 429.6 | 231.5 | 246.3 | 239.2 | 248.2 | 332.9 | 385.4 |
| 539 | 430.4 | 232.1 | 246.9 | 239.8 | 248.8 | 333.7 | 386.2 |
| 540 | 431.2 | 232.6 | 247.4 | 240.3 | 249.4 | 334.4 | 387.0 |
| 541 | 432.0 | 233.2 | 248.0 | 240.9 | 250.0 | 335.1 | 387.8 |
| 542 | 433.8 | 233.8 | 248.6 | 241.5 | 250.6 | 335.7 | 388.6 |
| 543 | 434.6 | 234.4 | 249.2 | 242.1 | 251.2 | 336.4 | 389.4 |

## TABLE* 17.

BROWN, MORRIS AND MILLAR'S TABLE FOR DETERMINING GLUCOSE, FRUCTOSE AND INVERT SUGAR.

| Milligrams of sugar. | Glucose. | | Fructose. | | Invert sugar. | |
|---|---|---|---|---|---|---|
| | Copper (Cu). | Cupric oxide (CuO). | Copper (Cu). | Cupric oxide (CuO). | Copper (Cu). | Cupric oxide (CuO). |
| | grams. | grams. | grams. | grams. | grams. | grams. |
| 50 | 0.1030 | 0.1289 | 0.0923 | 0.1155 | 0.0975 | 0.1221 |
| 55 | 0.1134 | 0.1422 | 0.1027 | 0.1287 | 0.1076 | 0.1349 |
| 60 | 0.1238 | 0.1552 | 0.1122 | 0.1407 | 0.1176 | 0.1474 |
| 65 | 0.1342 | 0.1682 | 0.1216 | 0.1524 | 0.1275 | 0.1598 |
| 70 | 0.1443 | 0.1809 | 0.1312 | 0.1645 | 0.1373 | 0.1721 |
| 75 | 0.1543 | 0.1935 | 0.1405 | 0.1761 | 0.1468 | 0.1840 |
| 80 | 0.1644 | 0.2061 | 0.1500 | 0.1881 | 0.1566 | 0.1963 |
| 85 | 0.1740 | 0.2187 | 0.1590 | 0.1993 | 0.1662 | 0.2084 |
| 90 | 0.1834 | 0.2299 | 0.1686 | 0.2114 | 0.1755 | 0.2200 |
| 95 | 0.1930 | 0.2420 | 0.1774 | 0.2224 | 0.1848 | 0.2317 |
| 100 | 0.2027 | 0.2538 | 0.1862 | 0.2331 | 0.1941 | 0.2430 |
| 105 | 0.2123 | 0.2662 | 0.1952 | 0.2447 | 0.2034 | 0.2550 |
| 110 | 0.2218 | 0.2781 | 0.2040 | 0.2558 | 0.2128 | 0.2668 |
| 115 | 0.2313 | 0.2900 | 0.2129 | 0.2669 | 0.2220 | 0.2783 |
| 120 | 0.2404 | 0.3014 | 0.2215 | 0.2777 | 0.2311 | 0.2898 |
| 125 | 0.2496 | 0.3130 | 0.2303 | 0.2887 | 0.2400 | 0.3009 |
| 130 | 0.2585 | 0.3241 | 0.2390 | 0.2997 | 0.2489 | 0.3121 |
| 135 | 0.2675 | 0.3354 | 0.2477 | 0.3106 | 0.2578 | 0.3232 |
| 140 | 0.2762 | 0.3463 | 0.2559 | 0.3209 | 0.2663 | 0.3339 |
| 145 | 0.2850 | 0.3573 | 0.2641 | 0.3311 | 0.2750 | 0.3448 |
| 150 | 0.2934 | 0.3673 | 0.2723 | 0.3409 | 0.2832 | 0.3546 |
| 155 | 0.3020 | 0.3787 | 0.2805 | 0.3517 | 0.2915 | 0.3655 |
| 160 | 0.3103 | 0.3891 | 0.2889 | 0.3622 | 0.3002 | 0.3764 |
| 165 | 0.3187 | 0.3996 | 0.2972 | 0.3726 | 0.3086 | 0 3869 |
| 170 | 0.3268 | 0.4098 | 0.3053 | 0.3828 | 0.3167 | 0.3971 |
| 175 | 0.3350 | 0.4200 | 0.3134 | 0.3930 | 0.3251 | 0.4076 |
| 180 | 0.3431 | 0.4302 | 0.3216 | 0.4032 | 0.3331 | 0.4177 |
| 185 | 0.3508 | 0.4399 | 0.3297 | 0.4134 | 0.3410 | 0.4276 |
| 190 | 0.3590 | 0.4501 | 0.3377 | 0.4234 | 0.3490 | 0.4376 |
| 195 | 0.3668 | 0.4599 | 0.3457 | 0.4335 | 0.3570 | 0.4476 |
| 200 | 0.3745 | 0.4689 | 0.3539 | 0.4431 | 0.3650 | 0.4570 |
| 205 | 0.3822 | 0.4792 | 0.3616 | 0.4534 | 0.3726 | 0.4672 |

* *See* "Handbook," page 425.

TABLE* 18.

DEFREN'S TABLE FOR DETERMINING GLUCOSE, MALTOSE AND LACTOSE.

| Cupric oxide. (CuO). | Glucose. | Maltose. | Lactose. | Cupric oxide. (CuO). | Glucose. | Maltose. | Lactose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| **30** | 13.2 | 21.7 | 18.8 | 83 | 36.8 | 60.3 | 52.4 |
| 31 | 13.7 | 22.4 | 19.5 | 84 | 37.2 | 61.1 | 53.0 |
| 32 | 14.1 | 23.1 | 20.1 | 85 | 37.7 | 61.8 | 53.6 |
| 33 | 14.6 | 23.9 | 20.7 | 86 | 38.1 | 62.5 | 54.3 |
| 34 | 15.0 | 24.6 | 21.4 | 87 | 38.5 | 63.3 | 54.9 |
| 35 | 15.4 | 25.3 | 22.0 | 88 | 39.0 | 64.0 | 55.5 |
| 36 | 15.9 | 26.1 | 22.6 | 89 | 39.4 | 64.7 | 56.2 |
| 37 | 16.3 | 26.8 | 23.3 | **90** | 39.9 | 65.5 | 56.8 |
| 38 | 16.8 | 27.5 | 23.9 | 91 | 40.3 | 66.2 | 57.4 |
| 39 | 17.2 | 28.3 | 24.5 | 92 | 40.8 | 66.9 | 58.1 |
| **40** | 17.6 | 29.0 | 25.2 | 93 | 41.2 | 67.7 | 58.7 |
| 41 | 18.1 | 29.7 | 25.8 | 94 | 41.7 | 68.4 | 59.3 |
| 42 | 18.5 | 30.5 | 26.4 | 95 | 42.1 | 69.1 | 60.0 |
| 43 | 19.0 | 31.2 | 27.1 | 96 | 42.5 | 69.9 | 60.6 |
| 44 | 19.4 | 31.9 | 27.7 | 97 | 43.0 | 70.6 | 61.2 |
| 45 | 19.9 | 32.7 | 28.3 | 98 | 43.4 | 71.3 | 61.9 |
| 46 | 20.3 | 33.4 | 29.0 | 99 | 43.9 | 72.1 | 62.5 |
| 47 | 20.7 | 34.1 | 29.6 | **100** | 44.4 | 72.8 | 63.2 |
| 48 | 21.2 | 34.8 | 30.2 | 101 | 44.8 | 73.5 | 63.8 |
| 49 | 21.6 | 35.5 | 30.8 | 102 | 45.3 | 74.3 | 64.4 |
| **50** | 22.1 | 36.2 | 31.5 | 103 | 45.7 | 75.0 | 65.1 |
| 51 | 22.5 | 37.0 | 32.1 | 104 | 46.2 | 75.7 | 65.7 |
| 52 | 23.0 | 37.7 | 32.7 | 105 | 46.6 | 76.5 | 66.3 |
| 53 | 23.4 | 38.4 | 33.3 | 106 | 47.0 | 77.2 | 67.0 |
| 54 | 23.8 | 39.2 | 34.0 | 107 | 47.5 | 77.9 | 67.6 |
| 55 | 24.2 | 39.9 | 34.6 | 108 | 48.0 | 78.7 | 68.2 |
| 56 | 24.7 | 40.5 | 35.2 | 109 | 48.4 | 79.4 | 68.9 |
| 57 | 25.1 | 41.3 | 35.9 | **110** | 48.9 | 80.1 | 69.5 |
| 58 | 25.5 | 42.1 | 36.5 | 111 | 49.3 | 80.9 | 70.1 |
| 59 | 26.0 | 42.8 | 37.1 | 112 | 49.8 | 81.6 | 70.8 |
| **60** | 26.4 | 43.5 | 37.8 | 113 | 50.2 | 82.3 | 71.4 |
| 61 | 26.9 | 44.3 | 38.4 | 114 | 50.7 | 83.1 | 72.0 |
| 62 | 27.3 | 45.0 | 39.0 | 115 | 51.1 | 83.8 | 72.7 |
| 63 | 27.8 | 45.7 | 39.7 | 116 | 51.6 | 84.5 | 73.3 |
| 64 | 28.2 | 46.5 | 40.3 | 117 | 52.0 | 85.2 | 74.0 |
| 65 | 28.7 | 47.2 | 40.9 | 118 | 52.4 | 85.9 | 74.6 |
| 66 | 29.1 | 47.9 | 41.6 | 119 | 52.9 | 86.6 | 75.2 |
| 67 | 29.5 | 48.6 | 42.2 | **120** | 53.3 | 87.4 | 75.9 |
| 68 | 30.0 | 49.4 | 42.8 | 121 | 53.8 | 88.1 | 76.6 |
| 69 | 30.4 | 50.1 | 43.5 | 122 | 54.2 | 88.9 | 77.2 |
| **70** | 30.9 | 50.8 | 44.1 | 123 | 54.7 | 89.6 | 77.9 |
| 71 | 31.3 | 51.6 | 44.7 | 124 | 55.1 | 90.3 | 78.5 |
| 72 | 31.8 | 52.3 | 45.4 | 125 | 55.6 | 91.1 | 79.1 |
| 73 | 32.2 | 53.0 | 46.0 | 126 | 56.0 | 91.8 | 79.8 |
| 74 | 32.6 | 53.8 | 46.6 | 127 | 56.5 | 92.5 | 80.4 |
| 75 | 33.1 | 54.5 | 47.3 | 128 | 56.9 | 93.3 | 81.1 |
| 76 | 33.5 | 55.2 | 47.9 | 129 | 57.3 | 94.0 | 81.7 |
| 77 | 34.0 | 56.0 | 48.5 | **130** | 57.8 | 94.8 | 82.4 |
| 78 | 34.4 | 56.7 | 49.2 | 131 | 58.2 | 95.5 | 83.0 |
| 79 | 34.9 | 57.4 | 49.8 | 132 | 58.7 | 96.2 | 83.6 |
| **80** | 35.4 | 58.1 | 50.5 | 133 | 59.1 | 97.0 | 84.2 |
| 81 | 35.9 | 58.9 | 51.1 | 134 | 59.6 | 97.7 | 84.9 |
| 82 | 36.3 | 59.6 | 51.7 | 135 | 60.0 | 98.4 | 85.5 |

* *See* "Handbook," page 425.

TABLE 18. (*Continued.*)

| Cupric oxide (CuO). | Glucose. | Maltose. | Lactose. | Cupric oxide (CuO). | Glucose. | Maltose. | Lactose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 136 | 60.5 | 99.2 | 86.1 | **190** | 84.9 | 139.1 | 121.0 |
| 137 | 60.9 | 99.9 | 86.8 | 191 | 85.4 | 139.9 | 121.7 |
| 138 | 61.3 | 100.7 | 87.4 | 192 | 85.9 | 140.6 | 122.3 |
| 139 | 61.8 | 101.4 | 88.1 | 193 | 86.3 | 141.4 | 123.0 |
| **140** | 62.2 | 102.1 | 88.7 | 194 | 86.8 | 142.1 | 123.6 |
| 141 | 62.7 | 102.8 | 89.3 | 195 | 87.2 | 142.8 | 124.3 |
| 142 | 63.1 | 103.5 | 90.0 | 196 | 87.7 | 143.6 | 124.9 |
| 143 | 63.6 | 104.3 | 90.6 | 197 | 88.1 | 144.3 | 125.6 |
| 144 | 64.0 | 105.0 | 91.3 | 198 | 88.6 | 145.1 | 126.2 |
| 145 | 64.5 | 105.8 | 91.9 | 199 | 89.0 | 145.8 | 126.9 |
| 146 | 64.9 | 106.5 | 92.6 | **200** | 89.5 | 146.6 | 127.5 |
| 147 | 65.4 | 107.2 | 93.2 | 201 | 89.9 | 147.3 | 128.2 |
| 148 | 65.8 | 108.0 | 93.9 | 202 | 90.4 | 148.1 | 128.8 |
| 149 | 66.3 | 108.7 | 94.5 | 203 | 90.8 | 148.8 | 129.5 |
| **150** | 66.8 | 109.5 | 95.2 | 204 | 91.3 | 149.6 | 130.1 |
| 151 | 67.3 | 110.2 | 95.8 | 205 | 91.7 | 150.3 | 130.8 |
| 152 | 67.7 | 111.0 | 96.5 | 206 | 92.2 | 151.1 | 131.5 |
| 153 | 68.3 | 111.7 | 97.1 | 207 | 92.6 | 151.8 | 132.1 |
| 154 | 68.7 | 112.4 | 97.8 | 208 | 93.1 | 152.5 | 132.8 |
| 155 | 69.2 | 113.2 | 98.4 | 209 | 93.5 | 153.3 | 133.4 |
| 156 | 69.6 | 113.9 | 99.1 | **210** | 94.0 | 154.1 | 134.1 |
| 157 | 70.0 | 114.7 | 99.7 | 211 | 94.4 | 154.8 | 134.7 |
| 158 | 70.5 | 115.4 | 100.4 | 212 | 94.9 | 155.6 | 135.4 |
| 159 | 70.9 | 116.1 | 101.0 | 213 | 95.3 | 156.3 | 136.0 |
| **160** | 71.3 | 116.9 | 101.7 | 214 | 95.8 | 157.1 | 136.7 |
| 161 | 71.8 | 117.6 | 102.3 | 215 | 96.3 | 157.8 | 137.3 |
| 162 | 72.3 | 118.4 | 103.0 | 216 | 96.7 | 158.6 | 138.0 |
| 163 | 72.7 | 119.1 | 103.6 | 217 | 97.2 | 159.3 | 138.6 |
| 164 | 73.2 | 119.9 | 104.3 | 218 | 97.6 | 160.0 | 139.3 |
| 165 | 73.6 | 120.6 | 104.9 | 219 | 98.1 | 160.8 | 139.9 |
| 166 | 74.1 | 121.4 | 105.6 | **220** | 98.6 | 161.5 | 140.6 |
| 167 | 74.5 | 122.1 | 106.2 | 221 | 99.0 | 162.3 | 141.2 |
| 168 | 74.9 | 122.9 | 106.9 | 222 | 99.5 | 163.0 | 141.9 |
| 169 | 75.4 | 123.6 | 107.5 | 223 | 99.9 | 163.7 | 142.5 |
| **170** | 75.8 | 124.4 | 108.2 | 224 | 100.4 | 164.5 | 143.2 |
| 171 | 76.3 | 125.1 | 108.8 | 225 | 100.9 | 165.3 | 143.8 |
| 172 | 76.8 | 125.8 | 109.5 | 226 | 101.3 | 166.0 | 144.5 |
| 173 | 77.3 | 126.6 | 110.1 | 227 | 101.8 | 166.8 | 145.1 |
| 174 | 77.7 | 127.3 | 110.8 | 228 | 102.2 | 167.5 | 145.8 |
| 175 | 78.2 | 128.1 | 111.4 | 229 | 102.7 | 168.3 | 146.4 |
| 176 | 78.6 | 128.8 | 112.0 | **230** | 103.1 | 169.1 | 147.0 |
| 177 | 79.1 | 129.5 | 112.6 | 231 | 103.6 | 169.8 | 147.7 |
| 178 | 79.5 | 130.3 | 113.3 | 232 | 104.0 | 170.6 | 148.3 |
| 179 | 80.0 | 131.0 | 113.9 | 233 | 104.5 | 171.3 | 149.0 |
| **180** | 80.4 | 131.8 | 114.6 | 234 | 105.0 | 172.1 | 149.6 |
| 181 | 80.8 | 132.5 | 115.2 | 235 | 105.4 | 172.8 | 150.3 |
| 182 | 81.3 | 133.2 | 115.8 | 236 | 105.9 | 173.6 | 150.9 |
| 183 | 81.8 | 134.0 | 116.5 | 237 | 106.3 | 174.3 | 151.6 |
| 184 | 82.2 | 134.7 | 117.1 | 238 | 106.8 | 175.1 | 152.2 |
| 185 | 82.7 | 135.5 | 117.8 | 239 | 107.2 | 175.8 | 152.9 |
| 186 | 83.1 | 136.2 | 118.4 | **240** | 107.7 | 176.6 | 153.5 |
| 187 | 83.5 | 136.9 | 119.1 | 241 | 108.1 | 177.3 | 154.2 |
| 188 | 84.0 | 137.7 | 119.7 | 242 | 108.6 | 178.1 | 154.8 |
| 189 | 84.4 | 138.4 | 120.4 | 243 | 109.0 | 178.8 | 155.5 |

TABLE 18. (*Concluded.*)

| Cupric oxide (CuO). | Glucose. | Maltose. | Lactose. | Cupric oxide (CuO). | Glucose. | Maltose. | Lactose. |
|---|---|---|---|---|---|---|---|
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 244 | 109.5 | 179.6 | 156.1 | 283 | 127.4 | 209.0 | 181.5 |
| 245 | 109.9 | 180.3 | 156.8 | 284 | 127.9 | 209.8 | 182.2 |
| 246 | 110.4 | 181.1 | 157.4 | 285 | 128.3 | 210.5 | 182.9 |
| 247 | 110.9 | 181.8 | 158.1 | 286 | 128.8 | 211.3 | 183.6 |
| 248 | 111.3 | 182.6 | 158.7 | 287 | 129.3 | 212.1 | 184.2 |
| 249 | 111.8 | 183.3 | 159.4 | 288 | 129.7 | 212.8 | 184.9 |
| **250** | 112.3 | 184.1 | 160.0 | 289 | 130.2 | 213.6 | 185.6 |
| 251 | 112.7 | 184.8 | 160.7 | **290** | 130.6 | 214.3 | 186.2 |
| 252 | 113.2 | 185.5 | 161.3 | 291 | 131.1 | 215.1 | 186.9 |
| 253 | 113.7 | 186.3 | 162.0 | 292 | 131.5 | 215.9 | 187.6 |
| 254 | 114.1 | 187.1 | 162.6 | 293 | 132.0 | 216.6 | 188.2 |
| 255 | 114.6 | 187.8 | 163.3 | 294 | 132.5 | 217.4 | 188.9 |
| 256 | 115.0 | 188.6 | 163.9 | 295 | 133.0 | 218.2 | 189.5 |
| 257 | 115.5 | 189.3 | 164.6 | 296 | 133.4 | 218.9 | 190.2 |
| 258 | 116.0 | 190.1 | 165.2 | 297 | 133.9 | 219.7 | 190.8 |
| 259 | 116.4 | 190.8 | 165.9 | 298 | 134.3 | 220.4 | 191.5 |
| **260** | 116.9 | 191.6 | 166.5 | 299 | 134.8 | 221.2 | 192.1 |
| 261 | 117.3 | 192.4 | 167.2 | **300** | 135.3 | 221.9 | 192.8 |
| 262 | 117.8 | 193.1 | 167.8 | 301 | 135.7 | 222.7 | 193.4 |
| 263 | 118.3 | 193.9 | 168.1 | 302 | 136.2 | 223.5 | 194.1 |
| 264 | 118.7 | 194.6 | 169.5 | 303 | 136.6 | 224.2 | 194.7 |
| 265 | 119.2 | 195.4 | 169.8 | 304 | 137.1 | 225.0 | 195.3 |
| 266 | 119.6 | 196.1 | 170.4 | 305 | 137.6 | 225.8 | 196.0 |
| 267 | 120.1 | 196.9 | 171.1 | 306 | 138.0 | 226.5 | 196.6 |
| 268 | 120.6 | 197.7 | 171.7 | 307 | 138.5 | 227.3 | 197.3 |
| 269 | 121.0 | 198.4 | 172.4 | 308 | 138.9 | 228.1 | 197.9 |
| **270** | 121.4 | 199.2 | 173.0 | 309 | 139.4 | 228.8 | 198.6 |
| 271 | 121.9 | 199.9 | 173.7 | **310** | 139.9 | 229.6 | 199.3 |
| 272 | 122.4 | 200.7 | 174.4 | 311 | 140.3 | 230.4 | 199.9 |
| 273 | 122.8 | 201.5 | 175.0 | 312 | 140.8 | 231.1 | 200.6 |
| 274 | 123.3 | 202.2 | 175.7 | 313 | 141.2 | 231.9 | 201.3 |
| 275 | 123.7 | 203.0 | 176.3 | 314 | 141.7 | 232.7 | 202.0 |
| 276 | 124.2 | 203.7 | 177.0 | 315 | 142.2 | 233.4 | 202.6 |
| 277 | 124.6 | 204.5 | 177.6 | 316 | 142.6 | 234.2 | 203.3 |
| 278 | 125.1 | 205.2 | 178.3 | 317 | 143.1 | 234.9 | 203.9 |
| 279 | 125.6 | 206.0 | 178.9 | 318 | 143.6 | 235.7 | 204.6 |
| **280** | 126.1 | 206.8 | 179.6 | 319 | 144.0 | 236.5 | 205.3 |
| 281 | 126.5 | 207.5 | 180.2 | **320** | 144.5 | 237.2 | 205.9 |
| 282 | 127.0 | 208.3 | 180.9 | | | | |

## TABLE* 19.

MUNSON AND WALKER'S TABLE FOR DETERMINING GLUCOSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND ½ GRAMS TOTAL SUGAR), LACTOSE AND MALTOSE.

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 10 | 8.9 | 4.0 | 4.5 | 1.6 | ..... | 3.8 | 4.0 | 5.9 | 6.2 |
| 11 | 9.8 | 4.5 | 5.0 | 2.1 | ..... | 4.5 | 4.7 | 6.7 | 7.0 |
| 12 | 10.7 | 4.9 | 5.4 | 2.5 | ..... | 5.1 | 5.4 | 7.5 | 7.9 |
| 13 | 11.5 | 5.3 | 5.8 | 3.0 | ..... | 5.8 | 6.1 | 8.3 | 8.7 |
| 14 | 12.4 | 5.7 | 6.3 | 3.4 | ..... | 6.4 | 6.8 | 9.1 | 9.5 |
| 15 | 13.3 | 6.2 | 6.7 | 3.9 | ..... | 7.1 | 7.5 | 9.9 | 10.4 |
| 16 | 14.2 | 6.6 | 7.2 | 4.3 | ..... | 7.8 | 8.2 | 10.6 | 11.2 |
| 17 | 15.1 | 7.0 | 7.6 | 4.8 | ..... | 8.4 | 8.9 | 11.4 | 12.0 |
| 18 | 16.0 | 7.5 | 8.1 | 5.2 | ..... | 9.1 | 9.5 | 12.2 | 12.9 |
| 19 | 16.9 | 7.9 | 8.5 | 5.7 | ..... | 9.7 | 10.2 | 13.0 | 13.7 |
| 20 | 17.8 | 8.3 | 8.9 | 6.1 | ..... | 10.4 | 10.9 | 13.8 | 14.6 |
| 21 | 18.7 | 8.7 | 9.4 | 6.6 | ..... | 11.0 | 11.6 | 14.6 | 15.4 |
| 22 | 19.5 | 9.2 | 9.8 | 7.0 | ..... | 11.7 | 12.3 | 15.4 | 16.2 |
| 23 | 20.4 | 9.6 | 10.3 | 7.5 | ..... | 12.3 | 13.0 | 16.2 | 17.1 |
| 24 | 21.3 | 10.0 | 10.7 | 7.9 | ..... | 13 0 | 13.7 | 17.0 | 17.9 |
| 25 | 22.2 | 10.5 | 11.2 | 8.4 | ..... | 13.7 | 14.4 | 17.8 | 18.7 |
| 26 | 23.1 | 10.9 | 11.6 | 8.8 | ..... | 14.3 | 15.1 | 18.6 | 19.6 |
| 27 | 24.0 | 11.3 | 12.0 | 9.3 | ..... | 15.0 | 15.8 | 19.4 | 20.4 |
| 28 | 24.9 | 11.8 | 12.5 | 9.7 | ..... | 15.6 | 16.5 | 20.2 | 21.2 |
| 29 | 25.8 | 12.2 | 12.9 | 10.2 | ..... | 16.3 | 17.1 | 21.0 | 22.1 |
| 30 | 26.6 | 12.6 | 13.4 | 10.7 | 4.3 | 16.9 | 17.8 | 21.8 | 22.9 |
| 31 | 27.5 | 13.1 | 13.8 | 11.1 | 4.7 | 17.6 | 18.5 | 22.6 | 23.7 |
| 32 | 28.4 | 13.5 | 14.3 | 11.6 | 5.2 | 18.3 | 19.2 | 23.3 | 24.6 |
| 33 | 29.3 | 13.9 | 14.7 | 12.0 | 5.6 | 18.9 | 19.9 | 24.1 | 25.4 |
| 34 | 30.2 | 14.3 | 15.2 | 12.5 | 6.1 | 19.6 | 20.6 | 24.9 | 26.2 |
| 35 | 31.1 | 14.8 | 15.6 | 12.9 | 6.5 | 20.2 | 21.3 | 25.7 | 27.1 |
| 36 | 32.0 | 15.2 | 16.1 | 13.4 | 7.0 | 20.9 | 22.0 | 26.5 | 27.9 |
| 37 | 32.9 | 15.6 | 16.5 | 13.8 | 7.4 | 21.5 | 22.7 | 27.3 | 28.7 |
| 38 | 33.8 | 16.1 | 16.9 | 14.3 | 7.9 | 22.2 | 23.4 | 28.1 | 29.6 |
| 39 | 34.6 | 16.5 | 17.4 | 14.7 | 8.4 | 22.8 | 24.1 | 28.9 | 30.4 |
| 40 | 35.5 | 16.9 | 17.8 | 15.2 | 8.8 | 23.5 | 24.8 | 29.7 | 31.3 |
| 41 | 36.4 | 17.4 | 18.3 | 15.6 | 9.3 | 24.2 | 25.4 | 30.5 | 32.1 |
| 42 | 37.3 | 17.8 | 18.7 | 16.1 | 9.7 | 24.8 | 26.1 | 31.3 | 32.9 |
| 43 | 38.2 | 18.2 | 19.2 | 16.6 | 10.2 | 25.5 | 26.8 | 32.1 | 33.8 |
| 44 | 39.1 | 18.7 | 19.6 | 17.0 | 10.7 | 26.1 | 27.5 | 32.9 | 34.6 |

* *See* "Handbook," page 426.

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar. and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 45 | 40.0 | 19.1 | 20.1 | 17.5 | 11.1 | 26.8 | 28.2 | 33.7 | 35.4 |
| 46 | 40.9 | 19.6 | 20.5 | 17.9 | 11.6 | 27.4 | 28.9 | 34.4 | 36.3 |
| 47 | 41.7 | 20.0 | 21.0 | 18.4 | 12.0 | 28.1 | 29.6 | 35.2 | 37.1 |
| 48 | 42.6 | 20.4 | 21.4 | 18.8 | 12.5 | 28.7 | 30.3 | 36.0 | 37.9 |
| 49 | 43.5 | 20.9 | 21.9 | 19.3 | 12.9 | 29.4 | 31.0 | 36.8 | 38.8 |
| 50 | 44.4 | 21.3 | 22.3 | 19.7 | 13.4 | 30.1 | 31.7 | 37.6 | 39.6 |
| 51 | 45.3 | 21.7 | 22.8 | 20.2 | 13.9 | 30.7 | 32.4 | 38.4 | 40.4 |
| 52 | 46.2 | 22.2 | 23.2 | 20.7 | 14.3 | 31.4 | 33.0 | 39.2 | 41.3 |
| 53 | 47.1 | 22.6 | 23.7 | 21.1 | 14.8 | 32.1 | 33.7 | 40.0 | 42.1 |
| 54 | 48.0 | 23.0 | 24.1 | 21.6 | 15.2 | 32.7 | 34.4 | 40.8 | 42.9 |
| 55 | 48.9 | 23.5 | 24.6 | 22.0 | 15.7 | 33.4 | 35.1 | 41.6 | 43.8 |
| 56 | 49.7 | 23.9 | 25.0 | 22.5 | 16.2 | 34.0 | 35.8 | 42.4 | 44.6 |
| 57 | 50.6 | 24.3 | 25.5 | 22.9 | 16.6 | 34.7 | 36.5 | 43.2 | 45.4 |
| 58 | 51.5 | 24.8 | 25.9 | 23.4 | 17.1 | 35.4 | 37.2 | 44.0 | 46.3 |
| 59 | 52.4 | 25.2 | 26.4 | 23.9 | 17.5 | 36.0 | 37.9 | 44.8 | 47.1 |
| 60 | 53.3 | 25.6 | 26.8 | 24.3 | 18.0 | 36.7 | 38.6 | 45.6 | 48.0 |
| 61 | 54.2 | 26.1 | 27.3 | 24.8 | 18.5 | 37.3 | 39.3 | 46.3 | 48.8 |
| 62 | 55.1 | 26.5 | 27.7 | 25.2 | 18.9 | 38.0 | 40.0 | 47.1 | 49.6 |
| 63 | 56.0 | 27.0 | 28.2 | 25.7 | 19.4 | 38.6 | 40.7 | 47.9 | 50.5 |
| 64 | 56.8 | 27.4 | 28.6 | 26.2 | 19.8 | 39.3 | 41.4 | 48.7 | 51.3 |
| 65 | 57.7 | 27.8 | 29.1 | 26.6 | 20.3 | 40.0 | 42.1 | 49.5 | 52.1 |
| 66 | 58.6 | 28.3 | 29.5 | 27.1 | 20.8 | 40.6 | 42.8 | 50.3 | 53.0 |
| 67 | 59.5 | 28.7 | 30.0 | 27.5 | 21.2 | 41.3 | 43.5 | 51.1 | 53.8 |
| 68 | 60.4 | 29.2 | 30.4 | 28.0 | 21.7 | 41.9 | 44.2 | 51.9 | 54.6 |
| 69 | 61.3 | 29.6 | 30.9 | 28.5 | 22.2 | 42.6 | 44.8 | 52.7 | 55.5 |
| 70 | 62.2 | 30.0 | 31.3 | 28.9 | 22.6 | 43.3 | 45.5 | 53.5 | 56.3 |
| 71 | 63.1 | 30.5 | 31.8 | 29.4 | 23.1 | 43.9 | 46.2 | 54.3 | 57.1 |
| 72 | 64.0 | 30.9 | 32.3 | 29.8 | 23.5 | 44.6 | 46.9 | 55.1 | 58.0 |
| 73 | 64.8 | 31.4 | 32.7 | 30.3 | 24.0 | 45.2 | 47.6 | 55.9 | 58.8 |
| 74 | 65.7 | 31.8 | 33.2 | 30.8 | 24.5 | 45.9 | 48.3 | 56.7 | 59.6 |
| 75 | 66.6 | 32.2 | 33.6 | 31.2 | 24.9 | 46.6 | 49.0 | 57.5 | 60.5 |
| 76 | 67.5 | 32.7 | 34.1 | 31.7 | 25.4 | 47.2 | 49.7 | 58.2 | 61.3 |
| 77 | 68.4 | 33.1 | 34.5 | 32.1 | 25.9 | 47.9 | 50.4 | 59.0 | 62.1 |
| 78 | 69.3 | 33.6 | 35.0 | 32.6 | 26.3 | 48.5 | 51.1 | 59.8 | 63.0 |
| 79 | 70.2 | 34.0 | 35.4 | 33.1 | 26.8 | 49.2 | 51.8 | 60.6 | 63.8 |
| 80 | 71.1 | 34.4 | 35.9 | 33.5 | 27.3 | 49.9 | 52.5 | 61.4 | 64.6 |
| 81 | 71.9 | 34.9 | 36.3 | 34.0 | 27.7 | 50.5 | 53.2 | 62.2 | 65.5 |
| 82 | 72.8 | 35.3 | 36.8 | 34.5 | 28.2 | 51.2 | 53.9 | 63.0 | 66.3 |
| 83 | 73.7 | 35.8 | 37.3 | 34.9 | 28.6 | 51.8 | 54.6 | 63.8 | 67.1 |
| 84 | 74.6 | 36.2 | 37.7 | 35.4 | 29.1 | 52.5 | 55.3 | 64.6 | 68.0 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 85 | 75.5 | 36.7 | 38.2 | 35.8 | 29.6 | 53.1 | 56.0 | 65.4 | 68.8 |
| 86 | 76.4 | 37.1 | 38.6 | 36.3 | 30.0 | 53.8 | 56.6 | 66.2 | 69.7 |
| 87 | 77.3 | 37.5 | 39.1 | 36.8 | 30.5 | 54.5 | 57.3 | 67.0 | 70.5 |
| 88 | 78.2 | 38.0 | 39.5 | 37.2 | 31.0 | 55.1 | 58.0 | 67.8 | 71.3 |
| 89 | 79.1 | 38.4 | 40.0 | 37.7 | 31.4 | 55.8 | 58.7 | 68.5 | 72.2 |
| 90 | 79.9 | 38.9 | 40.4 | 38.2 | 31.9 | 56.4 | 59.4 | 69.3 | 73.0 |
| 91 | 80.8 | 39.3 | 40.9 | 38.6 | 32.4 | 57.1 | 60.1 | 70.1 | 73.8 |
| 92 | 81.7 | 39.8 | 41.4 | 39.1 | 32.8 | 57.8 | 60.8 | 70.9 | 74.7 |
| 93 | 82.6 | 40.2 | 41.8 | 39.6 | 33.3 | 58.4 | 61.5 | 71.7 | 75.5 |
| 94 | 83.5 | 40.6 | 42.3 | 40.0 | 33.8 | 59.1 | 62.2 | 72.5 | 76.3 |
| 95 | 84.4 | 41.1 | 42.7 | 40.5 | 34.2 | 59.7 | 62.9 | 73.3 | 77.2 |
| 96 | 85.3 | 41.5 | 43.2 | 41.0 | 34.7 | 60.4 | 63.6 | 74.1 | 78.0 |
| 97 | 86.2 | 42.0 | 43.7 | 41.4 | 35.2 | 61.1 | 64.3 | 74.9 | 78.8 |
| 98 | 87.1 | 42.4 | 44.1 | 41.9 | 35.6 | 61.7 | 65.0 | 75.7 | 79.7 |
| 99 | 87.9 | 42.9 | 44.6 | 42.4 | 36.1 | 62.4 | 65.7 | 76.5 | 80.5 |
| 100 | 88.8 | 43.3 | 45.0 | 42.8 | 36.6 | 63.0 | 66.4 | 77.3 | 81.3 |
| 101 | 89.7 | 43.8 | 45.5 | 43.3 | 37.0 | 63.7 | 67.1 | 78.1 | 82.2 |
| 102 | 90.6 | 44.2 | 46.0 | 43.8 | 37.5 | 64.4 | 67.8 | 78.8 | 83.0 |
| 103 | 91.5 | 44.7 | 46.4 | 44.2 | 38.0 | 65.0 | 68.5 | 79.6 | 83.8 |
| 104 | 92.4 | 45.1 | 46.9 | 44.7 | 38.5 | 65.7 | 69.1 | 80.4 | 84.7 |
| 105 | 93.3 | 45.5 | 47.3 | 45.2 | 38.9 | 66.4 | 69.8 | 81.2 | 85.5 |
| 106 | 94.2 | 46.0 | 47.8 | 45.6 | 39.4 | 67.0 | 70.5 | 82.0 | 86.3 |
| 107 | 95.0 | 46.4 | 48.3 | 46.1 | 39.9 | 67.7 | 71.2 | 82.8 | 87.2 |
| 108 | 95.9 | 46.9 | 48.7 | 46.6 | 40.3 | 68.3 | 71.9 | 83.6 | 88.0 |
| 109 | 96.8 | 47.3 | 49.2 | 47.0 | 40.8 | 69.0 | 72.6 | 84.4 | 88.8 |
| 110 | 97.7 | 47.8 | 49.6 | 47.5 | 41.3 | 69.7 | 73.3 | 85.2 | 89.7 |
| 111 | 98.6 | 48.2 | 50.1 | 48.0 | 41.7 | 70.3 | 74.0 | 86.0 | 90.5 |
| 112 | 99.5 | 48.7 | 50.6 | 48.4 | 42.2 | 71.0 | 74.7 | 86.8 | 91.3 |
| 113 | 100.4 | 49.1 | 51.0 | 48.9 | 42.7 | 71.6 | 75.4 | 87.6 | 92.2 |
| 114 | 101.3 | 49.6 | 51.5 | 49.4 | 43.2 | 72.3 | 76.1 | 88.4 | 93.0 |
| 115 | 102.2 | 50.0 | 51.9 | 49.8 | 43.6 | 73.0 | 76.8 | 89.2 | 93.9 |
| 116 | 103.0 | 50.5 | 52.4 | 50.3 | 44.1 | 73.6 | 77.5 | 90.0 | 94.7 |
| 117 | 103.9 | 50.9 | 52.9 | 50.8 | 44.6 | 74.3 | 78.2 | 90.7 | 95.5 |
| 118 | 104.8 | 51.4 | 53.3 | 51.2 | 45.0 | 75.0 | 78.9 | 91.5 | 96.4 |
| 119 | 105.7 | 51.8 | 53.8 | 51.7 | 45.5 | 75.6 | 79.6 | 92.3 | 97.2 |
| 120 | 106.6 | 52.3 | 54.3 | 52.2 | 46.0 | 76.3 | 80.3 | 93.1 | 98.0 |
| 121 | 107.5 | 52.7 | 54.7 | 52.7 | 46.5 | 76.9 | 81.0 | 93.9 | 98.9 |
| 122 | 108.4 | 53.2 | 55.2 | 53.1 | 46.9 | 77.6 | 81.7 | 94.7 | 99.7 |
| 123 | 109.3 | 53.6 | 55.7 | 53.6 | 47.4 | 78.3 | 82.4 | 95.5 | 100.5 |
| 124 | 110.1 | 54.1 | 56.1 | 54.1 | 47.9 | 78.9 | 83.1 | 96.3 | 101.4 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 125 | 111.0 | 54.5 | 56.6 | 54.5 | 48.3 | 79.6 | 83.8 | 97.1 | 102.2 |
| 126 | 111.9 | 55.0 | 57.0 | 55.0 | 48.8 | 80.3 | 84.5 | 97.9 | 103.0 |
| 127 | 112.8 | 55.4 | 57.5 | 55.5 | 49.3 | 80.9 | 85.2 | 98.7 | 103.9 |
| 128 | 113.7 | 55.9 | 58.0 | 55.9 | 49.8 | 81.6 | 85.9 | 99.4 | 104.7 |
| 129 | 114.6 | 56.3 | 58.4 | 56.4 | 50.2 | 82.2 | 86.6 | 100.2 | 105.5 |
| 130 | 115.5 | 56.8 | 58.9 | 56.9 | 50.7 | 82.9 | 87.3 | 101.0 | 106.4 |
| 131 | 116.4 | 57.2 | 59.4 | 57.4 | 51.2 | 83.6 | 88.0 | 101.8 | 107.2 |
| 132 | 117.3 | 57.7 | 59.8 | 57.8 | 51.7 | 84.2 | 88.7 | 102.6 | 108.0 |
| 133 | 118.1 | 58.1 | 60.3 | 58.3 | 52.1 | 84.9 | 89.4 | 103.4 | 108.9 |
| 134 | 119.0 | 58.6 | 60.8 | 58.8 | 52.6 | 85.5 | 90.1 | 104.2 | 109.7 |
| 135 | 119.9 | 59.0 | 61.2 | 59.3 | 53.1 | 86.2 | 90.8 | 105.0 | 110.5 |
| 136 | 120.8 | 59.5 | 61.7 | 59.7 | 53.6 | 86.9 | 91.5 | 105.8 | 111.4 |
| 137 | 121.7 | 60.0 | 62.2 | 60.2 | 54.0 | 87.5 | 92.1 | 106.6 | 112.2 |
| 138 | 122.6 | 60.4 | 62.6 | 60.7 | 54.5 | 88.2 | 92.8 | 107.4 | 113.0 |
| 139 | 123.5 | 60.9 | 63.1 | 61.2 | 55.0 | 88.9 | 93.5 | 108.2 | 113.9 |
| 140 | 124.4 | 61.3 | 63.6 | 61.6 | 55.5 | 89.5 | 94.2 | 109.0 | 114.7 |
| 141 | 125.2 | 61.8 | 64.0 | 62.1 | 55.9 | 90.2 | 94.9 | 109.8 | 115.5 |
| 142 | 126.1 | 62.2 | 64.5 | 62.6 | 56.4 | 90.8 | 95.6 | 110.5 | 116.4 |
| 143 | 127.0 | 62.7 | 65.0 | 63.1 | 56.9 | 91.5 | 96.3 | 111.3 | 117.2 |
| 144 | 127.9 | 63.1 | 65.4 | 63.5 | 57.4 | 92.2 | 97.0 | 112 1 | 118.0 |
| 145 | 128.8 | 63.6 | 65.9 | 64.0 | 57.8 | 92.8 | 97.7 | 112.9 | 118.9 |
| 146 | 129.7 | 64.0 | 66.4 | 64.5 | 58.3 | 93.5 | 98.4 | 113.7 | 119.7 |
| 147 | 130.6 | 64.5 | 66.9 | 65.0 | 58.8 | 94.2 | 99.1 | 114.5 | 120.5 |
| 148 | 131.5 | 65.0 | 67.3 | 65.4 | 59.3 | 94.8 | 99.8 | 115.3 | 121.4 |
| 149 | 132.4 | 65.4 | 67.8 | 65.9 | 59.7 | 95.5 | 100.5 | 116.1 | 122.2 |
| 150 | 133.2 | 65.9 | 68.3 | 66.4 | 60.2 | 96.1 | 101.2 | 116.9 | 123.0 |
| 151 | 134.1 | 66.3 | 68.7 | 66.9 | 60.7 | 96.8 | 101.9 | 117.7 | 123.9 |
| 152 | 135.0 | 66.8 | 69.2 | 67.3 | 61.2 | 97.5 | 102.6 | 118.5 | 124.7 |
| 153 | 135.9 | 67.2 | 69.7 | 67.8 | 61.7 | 98.1 | 103.3 | 119.3 | 125.5 |
| 154 | 136.8 | 67.7 | 70.1 | 68.3 | 62.1 | 98.8 | 104.0 | 120.0 | 126.4 |
| 155 | 137.7 | 68.2 | 70.6 | 68.8 | 62.6 | 99.5 | 104.7 | 120.8 | 127.2 |
| 156 | 138.6 | 68.6 | 71.1 | 69.2 | 63.1 | 100.1 | 105.4 | 121.6 | 128.0 |
| 157 | 139.5 | 69.1 | 71.6 | 69.7 | 63.6 | 100.8 | 106.1 | 122.4 | 128.9 |
| 158 | 140.3 | 69.5 | 72.0 | 70.2 | 64.1 | 101.5 | 106.8 | 123.2 | 129.7 |
| 159 | 141.2 | 70.0 | 72.5 | 70.7 | 64.5 | 102.1 | 107.5 | 124.0 | 130.5 |
| 160 | 142.1 | 70.4 | 73.0 | 71.2 | 65.0 | 102.8 | 108.2 | 124.8 | 131.4 |
| 161 | 143.0 | 70.9 | 73.4 | 71.6 | 65.5 | 103.4 | 108.9 | 125.6 | 132.2 |
| 162 | 143.9 | 71.4 | 73.9 | 72.1 | 66.0 | 104.1 | 109.6 | 126.4 | 133.0 |
| 163 | 144.8 | 71.8 | 74.4 | 72.6 | 66.5 | 104.8 | 110.3 | 127.2 | 133.9 |
| 164 | 145.7 | 72.3 | 74.9 | 73.1 | 66.9 | 105.4 | 111.0 | 128.0 | 134.7 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 165 | 146.6 | 72.8 | 75.3 | 73.6 | 67.4 | 106.1 | 111.7 | 128.8 | 135.5 |
| 166 | 147.5 | 73.2 | 75.8 | 74.0 | 67.9 | 106.8 | 112.4 | 129.6 | 136.4 |
| 167 | 148.3 | 73.7 | 76.3 | 74.5 | 68.4 | 107.4 | 113.1 | 130.3 | 137.2 |
| 168 | 149.2 | 74.1 | 76.8 | 75.0 | 68.9 | 108.1 | 113.8 | 131.1 | 138.0 |
| 169 | 150.1 | 74.6 | 77.2 | 75.5 | 69.3 | 108.8 | 114.5 | 131.9 | 138.9 |
| 170 | 151.0 | 75.1 | 77.7 | 76.0 | 69.8 | 109.4 | 115.2 | 132.7 | 139.7 |
| 171 | 151.9 | 75.5 | 78.2 | 76.4 | 70.3 | 110.1 | 115.9 | 133.5 | 140.5 |
| 172 | 152.8 | 76.0 | 78.7 | 76.9 | 70.8 | 110.8 | 116.6 | 134.3 | 141.4 |
| 173 | 153.7 | 76.4 | 79.1 | 77.4 | 71.3 | 111.4 | 117.3 | 135.1 | 142.2 |
| 174 | 154.6 | 76.9 | 79.6 | 77.9 | 71.7 | 112.1 | 118.0 | 135.9 | 143.0 |
| 175 | 155.5 | 77.4 | 80.1 | 78.4 | 72.2 | 112.8 | 118.7 | 136.7 | 143.9 |
| 176 | 156.3 | 77.8 | 80.6 | 78.8 | 72.7 | 113.4 | 119.4 | 137.5 | 144.7 |
| 177 | 157.2 | 78.3 | 81.0 | 79.3 | 73.2 | 114.1 | 120.1 | 138.3 | 145.5 |
| 178 | 158.1 | 78.8 | 81.5 | 79.8 | 73.7 | 114.8 | 120.8 | 139.1 | 146.4 |
| 179 | 159.0 | 79.2 | 82.0 | 80.3 | 74.2 | 115.4 | 121.5 | 139.8 | 147.2 |
| 180 | 159.9 | 79.7 | 82.5 | 80.8 | 74.6 | 116.1 | 122.2 | 140.6 | 148.0 |
| 181 | 160.8 | 80.1 | 82.9 | 81.3 | 75.1 | 116.7 | 122.9 | 141.4 | 148.9 |
| 182 | 161.7 | 80.6 | 83.4 | 81.7 | 75.6 | 117.4 | 123.6 | 142.2 | 149.7 |
| 183 | 162.6 | 81.1 | 83.9 | 82.2 | 76.1 | 118.1 | 124.3 | 143.0 | 150.5 |
| 184 | 163.4 | 81.5 | 84.4 | 82.7 | 76.6 | 118.7 | 125.0 | 143.8 | 151.4 |
| 185 | 164.3 | 82.0 | 84.9 | 83.2 | 77.1 | 119.4 | 125.7 | 144.6 | 152.2 |
| 186 | 165.2 | 82.5 | 85.3 | 83.7 | 77.6 | 120.1 | 126.4 | 145.4 | 153.0 |
| 187 | 166.1 | 82.9 | 85.8 | 84.2 | 78.0 | 120.7 | 127.1 | 146.2 | 153.9 |
| 188 | 167.0 | 83.4 | 86.3 | 84.6 | 78.5 | 121.4 | 127.8 | 147.0 | 154.7 |
| 189 | 167.9 | 83.9 | 86.8 | 85.1 | 79.0 | 122.1 | 128.5 | 147.8 | 155.5 |
| 190 | 168.8 | 84.3 | 87.2 | 85.6 | 79.5 | 122.7 | 129.2 | 148.6 | 156.4 |
| 191 | 169.7 | 84.8 | 87.7 | 86.1 | 80.0 | 123.4 | 129.9 | 149.3 | 157.2 |
| 192 | 170.5 | 85.3 | 88.2 | 86.6 | 80.5 | 124.1 | 130.6 | 150.1 | 158.0 |
| 193 | 171.4 | 85.7 | 88.7 | 87.1 | 81.0 | 124.7 | 131.3 | 150.9 | 158.9 |
| 194 | 172.3 | 86.2 | 89.2 | 87.6 | 81.4 | 125.4 | 132.0 | 151.7 | 159.7 |
| 195 | 173.2 | 86.7 | 89.6 | 88.0 | 81.9 | 126.1 | 132.7 | 152.5 | 160.5 |
| 196 | 174.1 | 87.1 | 90.1 | 88.5 | 82.4 | 126.7 | 133.4 | 153.3 | 161.4 |
| 197 | 175.0 | 87.6 | 90.6 | 89.0 | 82.9 | 127.4 | 134.1 | 154.1 | 162.2 |
| 198 | 175.9 | 88.1 | 91.1 | 89.5 | 83.4 | 128.1 | 134.8 | 154.9 | 163.0 |
| 199 | 176.8 | 88.5 | 91.6 | 90.0 | 83.9 | 128.7 | 135.5 | 155.7 | 163.9 |
| 200 | 177.7 | 89.0 | 92.0 | 90.5 | 84.4 | 129.4 | 136.2 | 156.5 | 164.7 |
| 201 | 178.5 | 89.5 | 92.5 | 91.0 | 84.8 | 130.0 | 136.9 | 157.3 | 165.5 |
| 202 | 179.4 | 89.9 | 93.0 | 91.4 | 85.3 | 130.7 | 137.6 | 158.1 | 166.4 |
| 203 | 180.3 | 90.4 | 93.5 | 91.9 | 85.8 | 131.4 | 138.3 | 158.8 | 167.2 |
| 204 | 181.2 | 90.9 | 94.0 | 92.4 | 86.3 | 132.0 | 139.0 | 159.6 | 168.0 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 205 | 182.1 | 91.4 | 94.5 | 92.9 | 86.8 | 132.7 | 139.7 | 160.4 | 168.9 |
| 206 | 183.0 | 91.8 | 94.9 | 93.4 | 87.3 | 133.4 | 140.4 | 161.2 | 169.7 |
| 207 | 183.9 | 92.3 | 95.4 | 93.9 | 87.8 | 134.0 | 141.1 | 162.0 | 170.5 |
| 208 | 184.8 | 92.8 | 95.9 | 94.4 | 88.3 | 134.7 | 141.8 | 162.8 | 171.4 |
| 209 | 185.6 | 93.2 | 96.4 | 94.9 | 88.8 | 135.4 | 142.5 | 163.6 | 172.2 |
| 210 | 186.5 | 93.7 | 96.9 | 95.4 | 89.2 | 136.0 | 143.2 | 164.4 | 173.0 |
| 211 | 187.4 | 94.2 | 97.4 | 95.8 | 89.7 | 136.7 | 143.9 | 165.2 | 173.8 |
| 212 | 188.3 | 94.6 | 97.8 | 96.3 | 90.2 | 137.4 | 144.6 | 166.0 | 174.7 |
| 213 | 189.2 | 95.1 | 98.3 | 96.8 | 90.7 | 138.0 | 145.3 | 166.8 | 175.5 |
| 214 | 190.1 | 95.6 | 98.8 | 97.3 | 91.2 | 138.7 | 146.0 | 167.5 | 176.4 |
| 215 | 191.0 | 96.1 | 99.3 | 97.8 | 91.7 | 139.4 | 146.7 | 168.3 | 177.2 |
| 216 | 191.9 | 96.5 | 99.8 | 98.3 | 92.2 | 140.0 | 147.4 | 169.1 | 178.0 |
| 217 | 192.8 | 97.0 | 100.3 | 98.8 | 92.7 | 140.7 | 148.1 | 169.9 | 178.9 |
| 218 | 193.6 | 97.5 | 100.8 | 99.3 | 93.2 | 141.4 | 148.8 | 170.7 | 179.7 |
| 219 | 194.5 | 98.0 | 101.2 | 99.8 | 93.7 | 142.0 | 149.5 | 171.5 | 180.5 |
| 220 | 195.4 | 98.4 | 101.7 | 100.3 | 94.2 | 142.7 | 150.2 | 172.3 | 181.4 |
| 221 | 196.3 | 98.9 | 102.2 | 100.8 | 94.7 | 143.4 | 150.9 | 173.1 | 182.2 |
| 222 | 197.2 | 99.4 | 102.7 | 101.2 | 95.1 | 144.0 | 151.6 | 173.9 | 183.0 |
| 223 | 198.1 | 99.9 | 103.2 | 101.7 | 95.6 | 144.7 | 152.3 | 174.7 | 183.9 |
| 224 | 199.0 | 100.3 | 103.7 | 102.2 | 96.1 | 145.4 | 153.0 | 175.5 | 184.7 |
| 225 | 199.9 | 100.8 | 104.2 | 102.7 | 96.6 | 146.0 | 153.7 | 176.2 | 185.5 |
| 226 | 200.7 | 101.3 | 104.6 | 103.2 | 97.1 | 146.7 | 154.4 | 177.0 | 186.4 |
| 227 | 201.6 | 101.8 | 105.1 | 103.7 | 97.6 | 147.4 | 155.1 | 177.8 | 187.2 |
| 228 | 202.5 | 102.2 | 105.6 | 104.2 | 98.1 | 148.0 | 155.8 | 178.6 | 188.0 |
| 229 | 203.4 | 102.7 | 106.1 | 104.7 | 98.6 | 148.7 | 156.5 | 179.4 | 188.8 |
| 230 | 204.3 | 103.2 | 106.6 | 105.2 | 99.1 | 149.4 | 157.2 | 180.2 | 189.7 |
| 231 | 205.2 | 103.7 | 107.1 | 105.7 | 99.6 | 150.0 | 157.9 | 181.0 | 190.5 |
| 232 | 206.1 | 104.1 | 107.6 | 106.2 | 100.1 | 150.7 | 158.6 | 181.8 | 191.3 |
| 233 | 207.0 | 104.6 | 108.1 | 106.7 | 100.6 | 151.4 | 159.3 | 182.6 | 192.2 |
| 234 | 207.9 | 105.1 | 108.6 | 107.2 | 101.1 | 152.0 | 160.0 | 183.4 | 193.0 |
| 235 | 208.7 | 105.6 | 109.1 | 107.7 | 101.6 | 152.7 | 160.7 | 184.2 | 193.8 |
| 236 | 209.6 | 106.0 | 109.5 | 108.2 | 102.1 | 153.4 | 161.4 | 184.9 | 194.7 |
| 237 | 210.5 | 106.5 | 110.0 | 108.7 | 102.6 | 154.0 | 162.1 | 185.7 | 195.5 |
| 238 | 211.4 | 107.0 | 110.5 | 109.2 | 103.1 | 154.7 | 162.8 | 186.5 | 196.3 |
| 239 | 212.3 | 107.5 | 111.0 | 109.6 | 103.5 | 155.4 | 163.5 | 187.3 | 197.2 |
| 240 | 213.2 | 108.0 | 111.5 | 110.1 | 104.0 | 156.1 | 164.3 | 188.1 | 198.0 |
| 241 | 214.1 | 108.4 | 112.0 | 110.6 | 104.5 | 156.7 | 165.0 | 188.9 | 198.8 |
| 242 | 215.0 | 108.9 | 112.5 | 111.1 | 105.0 | 157.4 | 165.7 | 189.7 | 199.7 |
| 243 | 215.8 | 109.4 | 113.0 | 111.6 | 105.5 | 158.1 | 166.4 | 190.5 | 200.5 |
| 244 | 216.7 | 109.9 | 113.5 | 112.1 | 106.0 | 158.7 | 167.1 | 191.3 | 201.3 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 245 | 217.6 | 110.4 | 114.0 | 112.6 | 106.5 | 159.4 | 167.8 | 192.1 | 202.2 |
| 246 | 218.5 | 110.8 | 114.5 | 113.1 | 107.0 | 160.1 | 168.5 | 192.9 | 203.0 |
| 247 | 219.4 | 111.3 | 115.0 | 113.6 | 107.5 | 160.7 | 169.2 | 193.6 | 203.8 |
| 248 | 220.3 | 111.8 | 115.4 | 114.1 | 108.0 | 161.4 | 169.9 | 194.4 | 204.7 |
| 249 | 221.2 | 112.3 | 115.9 | 114.6 | 108.5 | 162.1 | 170.6 | 195.2 | 205.5 |
| 250 | 222.1 | 112.8 | 116.4 | 115.1 | 109.0 | 162.7 | 171.3 | 196.0 | 206.3 |
| 251 | 223.0 | 113.2 | 116.9 | 115.6 | 109.5 | 163.4 | 172.0 | 196.8 | 207.2 |
| 252 | 223.8 | 113.7 | 117.4 | 116.1 | 110.0 | 164.1 | 172.7 | 197.6 | 208.0 |
| 253 | 224.7 | 114.2 | 117.9 | 116.6 | 110.5 | 164.7 | 173.4 | 198.4 | 208.8 |
| 254 | 225.6 | 114.7 | 118.4 | 117.1 | 111.0 | 165.4 | 174.1 | 199.2 | 209.7 |
| 255 | 226.5 | 115.2 | 118.9 | 117.6 | 111.5 | 166.1 | 174.8 | 200.0 | 210.5 |
| 256 | 227.4 | 115.7 | 119.4 | 118.1 | 112.0 | 166.8 | 175.5 | 200.8 | 211.3 |
| 257 | 228.3 | 116.1 | 119.9 | 118.6 | 112.5 | 167.4 | 176.2 | 201.6 | 212.2 |
| 258 | 229.2 | 116.6 | 120.4 | 119.1 | 113.0 | 168.1 | 176.9 | 202.3 | 213.0 |
| 259 | 230.1 | 117.1 | 120.9 | 119.6 | 113.5 | 168.8 | 177.6 | 203.1 | 213.8 |
| 260 | 231.0 | 117.6 | 121.4 | 120.1 | 114.0 | 169.4 | 178.3 | 203.9 | 214.7 |
| 261 | 231.8 | 118.1 | 121.9 | 120.6 | 114.5 | 170.1 | 179.0 | 204.7 | 215.5 |
| 262 | 232.7 | 118.6 | 122.4 | 121.1 | 115.0 | 170.8 | 179.8 | 205.5 | 216.3 |
| 263 | 233.6 | 119.0 | 122.9 | 121.6 | 115.5 | 171.4 | 180.5 | 206.3 | 217.2 |
| 264 | 234.5 | 119.5 | 123.4 | 122.1 | 116.0 | 172.1 | 181.2 | 207.1 | 218.0 |
| 265 | 235.4 | 120.0 | 123.9 | 122.6 | 116.5 | 172.8 | 181.9 | 207.9 | 218.8 |
| 266 | 236.3 | 120.5 | 124.4 | 123.1 | 117.0 | 173.5 | 182.6 | 208.7 | 219.7 |
| 267 | 237.2 | 121.0 | 124.9 | 123.6 | 117.5 | 174.1 | 183.3 | 209.5 | 220.5 |
| 268 | 238.1 | 121.5 | 125.4 | 124.1 | 118.0 | 174.8 | 184.0 | 210.3 | 221.3 |
| 269 | 238.9 | 122.0 | 125.9 | 124.6 | 118.5 | 175.5 | 184.7 | 211.0 | 222.1 |
| 270 | 239.8 | 122.5 | 126.4 | 125.1 | 119.0 | 176.1 | 185.4 | 211.8 | 223.0 |
| 271 | 240.7 | 122.9 | 126.9 | 125.6 | 119.5 | 176.8 | 186.1 | 212.6 | 223.8 |
| 272 | 241.6 | 123.4 | 127.4 | 126.2 | 120.0 | 177.5 | 186.8 | 213.4 | 224.6 |
| 273 | 242.5 | 123.9 | 127.9 | 126.7 | 120.6 | 178.1 | 187.5 | 214.2 | 225.5 |
| 274 | 243.4 | 124.4 | 128.4 | 127.2 | 121.1 | 178.8 | 188.2 | 215.0 | 226.3 |
| 275 | 244.3 | 124.9 | 128.9 | 127.7 | 121.6 | 179.5 | 188.9 | 215.8 | 227.1 |
| 276 | 245.2 | 125.4 | 129.4 | 128.2 | 122.1 | 180.2 | 189.6 | 216.6 | 228.0 |
| 277 | 246.1 | 125.9 | 129.9 | 128.7 | 122.6 | 180.8 | 190.3 | 217.4 | 228.8 |
| 278 | 246.9 | 126.4 | 130.4 | 129.2 | 123.1 | 181.5 | 191.0 | 218.2 | 229.6 |
| 279 | 247.8 | 126.9 | 130.9 | 129.7 | 123.6 | 182.2 | 191.7 | 218.9 | 230.5 |
| 280 | 248.7 | 127.3 | 131.4 | 130.2 | 124.1 | 182.8 | 192.4 | 219.7 | 231.3 |
| 281 | 249.6 | 127.8 | 131.9 | 130.7 | 124.6 | 183.5 | 193.1 | 220.5 | 232.1 |
| 282 | 250.5 | 128.3 | 132.4 | 131.2 | 125.1 | 184.2 | 193.9 | 221.3 | 233.0 |
| 283 | 251.4 | 128.8 | 132.9 | 131.7 | 125.6 | 184.8 | 194.6 | 222.1 | 233.8 |
| 284 | 252.3 | 129.3 | 133.4 | 132.2 | 126.1 | 185.5 | 195.3 | 222.9 | 234.6 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 285 | 253.2 | 129.8 | 133.9 | 132.7 | 126.6 | 186.2 | 196.0 | 223.7 | 235.5 |
| 286 | 254.0 | 130.3 | 134.4 | 133.2 | 127.1 | 186.9 | 196.7 | 224.5 | 236.3 |
| 287 | 254.9 | 130.8 | 134.9 | 133.7 | 127.6 | 187.5 | 197.4 | 225.3 | 237.1 |
| 288 | 255.8 | 131.3 | 135.4 | 134.3 | 128.1 | 188.2 | 198.1 | 226.1 | 238.0 |
| 289 | 256.7 | 131.8 | 135.9 | 134.8 | 128.6 | 188.9 | 198.8 | 226.9 | 238.8 |
| 290 | 257.6 | 132.3 | 136.4 | 135.3 | 129.2 | 189.5 | 199.5 | 227.6 | 239.6 |
| 291 | 258.5 | 132.7 | 136.9 | 135.8 | 129.7 | 190.2 | 200.2 | 228.4 | 240.5 |
| 292 | 259.4 | 133.2 | 137.4 | 136.3 | 130.2 | 190.9 | 200.9 | 229.2 | 241.3 |
| 293 | 260.3 | 133.7 | 137.9 | 136.8 | 130.7 | 191.5 | 201.6 | 230.0 | 242.1 |
| 294 | 261.2 | 134.2 | 138.4 | 137.3 | 131.2 | 192.2 | 202.3 | 230.8 | 242.9 |
| 295 | 262.0 | 134.7 | 138.9 | 137.8 | 131.7 | 192.9 | 203.0 | 231.6 | 243.8 |
| 296 | 262.9 | 135.2 | 139.4 | 138.3 | 132.2 | 193.6 | 203.7 | 232.4 | 244.6 |
| 297 | 263.8 | 135.7 | 140.0 | 138.8 | 132.7 | 194.2 | 204.4 | 233.2 | 245.4 |
| 298 | 264.7 | 136.2 | 140.5 | 139.4 | 133.2 | 194.9 | 205.1 | 234.0 | 246.3 |
| 299 | 265.6 | 136.7 | 141.0 | 139.9 | 133.7 | 195.6 | 205.8 | 234.8 | 247.1 |
| 300 | 266.5 | 137.2 | 141.5 | 140.4 | 134.2 | 196.2 | 206.6 | 235.5 | 247.9 |
| 301 | 267.4 | 137.7 | 142.0 | 140.9 | 134.8 | 196.9 | 207.3 | 236.3 | 248.8 |
| 302 | 268.3 | 138.2 | 142.5 | 141.4 | 135.3 | 197.6 | 208.0 | 237.1 | 249.6 |
| 303 | 269.1 | 138.7 | 143.0 | 141.9 | 135.8 | 198.3 | 208.7 | 237.9 | 250.4 |
| 304 | 270.0 | 139.2 | 143.5 | 142.4 | 136.3 | 198.9 | 209.4 | 238.7 | 251.3 |
| 305 | 270.9 | 139.7 | 144.0 | 142.9 | 136.8 | 199.6 | 210.1 | 239.5 | 252.1 |
| 306 | 271.8 | 140.2 | 144.5 | 143.4 | 137.3 | 200.3 | 210.8 | 240.3 | 252.9 |
| 307 | 272.7 | 140.7 | 145.0 | 144.0 | 137.8 | 201.0 | 211.5 | 241.1 | 253.8 |
| 308 | 273.6 | 141.2 | 145.5 | 144.5 | 138.3 | 201.6 | 212.2 | 241.9 | 254.6 |
| 309 | 274.5 | 141.7 | 146.1 | 145.0 | 138.8 | 202.3 | 212.9 | 242.7 | 255.4 |
| 310 | 275.4 | 142.2 | 146.6 | 145.5 | 139.4 | 203.0 | 213.7 | 243.5 | 256.3 |
| 311 | 276.3 | 142.7 | 147.1 | 146.0 | 139.9 | 203.6 | 214.4 | 244.2 | 257.1 |
| 312 | 277.1 | 143.2 | 147.6 | 146.5 | 140.4 | 204.3 | 215.1 | 245.0 | 257.9 |
| 313 | 278.0 | 143.7 | 148.1 | 147.0 | 140.9 | 205.0 | 215.8 | 245.8 | 258.8 |
| 314 | 278.9 | 144.2 | 148.6 | 147.6 | 141.4 | 205.7 | 216.5 | 246.6 | 259.6 |
| 315 | 279.8 | 144.7 | 149.1 | 148.1 | 141.9 | 206.3 | 217.2 | 247.4 | 260.4 |
| 316 | 280.7 | 145.2 | 149.6 | 148.6 | 142.4 | 207.0 | 217.9 | 248.2 | 261.2 |
| 317 | 281.6 | 145.7 | 150.1 | 149.1 | 143.0 | 207.7 | 218.6 | 249.0 | 262.1 |
| 318 | 282.5 | 146.2 | 150.7 | 149.6 | 143.5 | 208.4 | 219.3 | 249.8 | 262.9 |
| 319 | 283.4 | 146.7 | 151.2 | 150.1 | 144.0 | 209.0 | 220.0 | 250.6 | 263.7 |
| 320 | 284.2 | 147.2 | 151.7 | 150.7 | 144.5 | 209.7 | 220.7 | 251.3 | 264.6 |
| 321 | 285.1 | 147.7 | 152.2 | 151.2 | 145.0 | 210.4 | 221.4 | 252.1 | 265.4 |
| 322 | 286.0 | 148.2 | 152.7 | 151.7 | 145.5 | 211.0 | 222.2 | 252.9 | 266.2 |
| 323 | 286.9 | 148.7 | 153.2 | 152.2 | 146.0 | 211.7 | 222.9 | 253.7 | 267.1 |
| 324 | 287.8 | 149.2 | 153.7 | 152.7 | 146.6 | 212.4 | 223.6 | 254.5 | 267.9 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 325 | 288.7 | 149.7 | 154.3 | 153.2 | 147.1 | 213.1 | 224.3 | 255.3 | 268.7 |
| 326 | 289.6 | 150.2 | 154.8 | 153.8 | 147.6 | 213.7 | 225.0 | 256.1 | 269.6 |
| 327 | 290.5 | 150.7 | 155.3 | 154.3 | 148.1 | 214.4 | 225.7 | 256.9 | 270.4 |
| 328 | 291.4 | 151.2 | 155.8 | 154.8 | 148.6 | 215.1 | 226.4 | 257.7 | 271.2 |
| 329 | 292.2 | 151.7 | 156.3 | 155.3 | 149.1 | 215.8 | 227.1 | 258.5 | 272.1 |
| 330 | 293.1 | 152.2 | 156.8 | 155.8 | 149.7 | 216.4 | 227.8 | 259.3 | 272.9 |
| 331 | 294.0 | 152.7 | 157.3 | 156.4 | 150.2 | 217.1 | 228.5 | 260.0 | 273.7 |
| 332 | 294.9 | 153.2 | 157.9 | 156.9 | 150.7 | 217.8 | 229.2 | 260.8 | 274.6 |
| 333 | 295.8 | 153.7 | 158.4 | 157.4 | 151.2 | 218.4 | 230.0 | 261.6 | 275.4 |
| 334 | 296.7 | 154.2 | 158.9 | 157.9 | 151.7 | 219.1 | 230.7 | 262.4 | 276.2 |
| 335 | 297.6 | 154.7 | 159.4 | 158.4 | 152.3 | 219.8 | 231.4 | 263.2 | 277.0 |
| 336 | 298.5 | 155.2 | 159.9 | 159.0 | 152.8 | 220.5 | 232.1 | 264.0 | 277.9 |
| 337 | 299.3 | 155.8 | 160.5 | 159.5 | 153.3 | 221.1 | 232.8 | 264.8 | 278.7 |
| 338 | 300.2 | 156.3 | 161.0 | 160.0 | 153.8 | 221.8 | 233.5 | 265.6 | 279.5 |
| 339 | 301.1 | 156.8 | 161.5 | 160.5 | 154.3 | 222.5 | 234.2 | 266.4 | 280.4 |
| 340 | 302.0 | 157.3 | 162.0 | 161.0 | 154.8 | 223.2 | 234.9 | 267.1 | 281.2 |
| 341 | 302.9 | 157.8 | 162.5 | 161.6 | 155.4 | 223.8 | 235.6 | 267.9 | 282.0 |
| 342 | 303.8 | 158.3 | 163.1 | 162.1 | 155.9 | 224.5 | 236.3 | 268.7 | 282.9 |
| 343 | 304.7 | 158.8 | 163.6 | 162.6 | 156.4 | 225.2 | 237.0 | 269.5 | 283.7 |
| 344 | 305.6 | 159.3 | 164.1 | 163.1 | 156.9 | 225.9 | 237.8 | 270.3 | 284.5 |
| 345 | 306.5 | 159.8 | 164.6 | 163.7 | 157.5 | 226.5 | 238.5 | 271.1 | 285.4 |
| 346 | 307.3 | 160.3 | 165.1 | 164.2 | 158.0 | 227.2 | 239.2 | 271.9 | 286.2 |
| 347 | 308.2 | 160.8 | 165.7 | 164.7 | 158.5 | 227.9 | 239.9 | 272.7 | 287.0 |
| 348 | 309.1 | 161.4 | 166.2 | 165.2 | 159.0 | 228.5 | 240.6 | 273.5 | 287.9 |
| 349 | 310.0 | 161.9 | 166.7 | 165.7 | 159.5 | 229.2 | 241.3 | 274.3 | 288.7 |
| 350 | 310.9 | 162.4 | 167.2 | 166.3 | 160.1 | 229.9 | 242.0 | 275.0 | 289.5 |
| 351 | 311.8 | 162.9 | 167.7 | 166.8 | 160.6 | 230.6 | 242.7 | 275.8 | 290.4 |
| 352 | 312.7 | 163.4 | 168.3 | 167.3 | 161.1 | 231.2 | 243.4 | 276.6 | 291.2 |
| 353 | 313.6 | 163.9 | 168.8 | 167.8 | 161.6 | 231.9 | 244.1 | 277.4 | 292.0 |
| 354 | 314.4 | 164.4 | 169.3 | 168.4 | 162.2 | 232.6 | 244.8 | 278.2 | 292.8 |
| 355 | 315.3 | 164.9 | 169.8 | 168.9 | 162.7 | 233.3 | 245.6 | 279.0 | 293.7 |
| 356 | 316.2 | 165.4 | 170.4 | 169.4 | 163.2 | 233.9 | 246.3 | 279.8 | 294.5 |
| 357 | 317.1 | 166.0 | 170.9 | 170.0 | 163.7 | 234.6 | 247.0 | 280.6 | 295.3 |
| 358 | 318.0 | 166.5 | 171.4 | 170.5 | 164.3 | 235.3 | 247.7 | 281.4 | 296.2 |
| 359 | 318.9 | 167.0 | 171.9 | 171.0 | 164.8 | 236.0 | 248.4 | 282.2 | 297.0 |
| 360 | 319.8 | 167.5 | 172.5 | 171.5 | 165.3 | 236.7 | 249.1 | 282.9 | 297.8 |
| 361 | 320.7 | 168.0 | 173.0 | 172.1 | 165.8 | 237.3 | 249.8 | 283.7 | 298.7 |
| 362 | 321.6 | 168.5 | 173.5 | 172.6 | 166.4 | 238.0 | 250.5 | 284.5 | 299.5 |
| 363 | 322.4 | 169.0 | 174.0 | 173.1 | 166.9 | 238.7 | 251.2 | 285.3 | 300.3 |
| 364 | 323.3 | 169.6 | 174.6 | 173.7 | 167.4 | 239.4 | 252.0 | 286.1 | 301.2 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 365 | 324.2 | 170.1 | 175.1 | 174.2 | 167.9 | 240.0 | 252.7 | 286.9 | 302.0 |
| 366 | 325.1 | 170.6 | 175.6 | 174.7 | 168.5 | 240.7 | 253.4 | 287.7 | 302.8 |
| 367 | 326.0 | 171.1 | 176.1 | 175.2 | 169.0 | 241.4 | 254.1 | 288.5 | 303.6 |
| 368 | 326.9 | 171.6 | 176.7 | 175.8 | 169.5 | 242.1 | 254.8 | 289.3 | 304.5 |
| 369 | 327.8 | 172.1 | 177.2 | 176.3 | 170.0 | 242.7 | 255.5 | 290.0 | 305.3 |
| 370 | 328.7 | 172.7 | 177.7 | 176.8 | 170.6 | 243.4 | 256.2 | 290.8 | 306.1 |
| 371 | 329.5 | 173.2 | 178.3 | 177.4 | 171.1 | 244.1 | 256.9 | 291.6 | 307.0 |
| 372 | 330.4 | 173.7 | 178.8 | 177.9 | 171.6 | 244.8 | 257.7 | 292.4 | 307.8 |
| 373 | 331.3 | 174.2 | 179.3 | 178.4 | 172.2 | 245.4 | 258.4 | 293.2 | 308.6 |
| 374 | 332.2 | 174.7 | 179.8 | 179.0 | 172.7 | 246.1 | 259.1 | 294.0 | 309.5 |
| 375 | 333.1 | 175.3 | 180.4 | 179.5 | 173.2 | 246.8 | 259.8 | 294.8 | 310.3 |
| 376 | 334.0 | 175.8 | 180.9 | 180.0 | 173.7 | 247.5 | 260.5 | 295.6 | 311.1 |
| 377 | 334.9 | 176.3 | 181.4 | 180.6 | 174.3 | 248.1 | 261.2 | 296.4 | 312.0 |
| 378 | 335.8 | 176.8 | 182.0 | 181.1 | 174.8 | 248.8 | 261.9 | 297.2 | 312.8 |
| 379 | 336.7 | 177.3 | 182.5 | 181.6 | 175.3 | 249.5 | 262.6 | 297.9 | 313.6 |
| 380 | 337.5 | 177.9 | 183.0 | 182.1 | 175.9 | 250.2 | 263.4 | 298.7 | 314.5 |
| 381 | 338.4 | 178.4 | 183.6 | 182.7 | 176.4 | 250.8 | 264.1 | 299.5 | 315.3 |
| 382 | 339.3 | 178.9 | 184.1 | 183.2 | 176.9 | 251.5 | 264.8 | 300.3 | 316.1 |
| 383 | 340.2 | 179.4 | 184.6 | 183.8 | 177.5 | 252.2 | 265.5 | 301.1 | 316.9 |
| 384 | 341.1 | 180.0 | 185.2 | 184.3 | 178.0 | 252.9 | 266.2 | 301.9 | 317.8 |
| 385 | 342.0 | 180.5 | 185.7 | 184.8 | 178.5 | 253.6 | 266.9 | 302.7 | 318.6 |
| 386 | 342.9 | 181.0 | 186.2 | 185.4 | 179.1 | 254.2 | 267.6 | 303.5 | 319.4 |
| 387 | 343.8 | 181.5 | 186.8 | 185.9 | 179.6 | 254.9 | 268.3 | 304.2 | 320.3 |
| 388 | 344.6 | 182.0 | 187.3 | 186.4 | 180.1 | 255.6 | 269.0 | 305.0 | 321.1 |
| 389 | 345.5 | 182.6 | 187.8 | 187.0 | 180.6 | 256.3 | 269.8 | 305.8 | 321.9 |
| 390 | 346.4 | 183.1 | 188.4 | 187.5 | 181.2 | 256.9 | 270.5 | 306.6 | 322.8 |
| 391 | 347.3 | 183.6 | 188.9 | 188.0 | 181.7 | 257.6 | 271.2 | 307.4 | 323.6 |
| 392 | 348.2 | 184.1 | 189.4 | 188.6 | 182.3 | 258.3 | 271.9 | 308.2 | 324.4 |
| 393 | 349.1 | 184.7 | 190.0 | 189.1 | 182.8 | 259.0 | 272.6 | 309.0 | 325.2 |
| 394 | 350.0 | 185.2 | 190.5 | 189.7 | 183.3 | 259.6 | 273.3 | 309.8 | 326.1 |
| 395 | 350.9 | 185.7 | 191.0 | 190.2 | 183.9 | 260.3 | 274.0 | 310.6 | 326.9 |
| 396 | 351.8 | 186.2 | 191.6 | 190.7 | 184.4 | 261.0 | 274.7 | 311.4 | 327.7 |
| 397 | 352.6 | 186.8 | 192.1 | 191.3 | 184.9 | 261.7 | 275.5 | 312.1 | 328.6 |
| 398 | 353.5 | 187.3 | 192.7 | 191.8 | 185.5 | 262.3 | 276.2 | 312.9 | 329.4 |
| 399 | 354.4 | 187.8 | 193.2 | 192.3 | 186.0 | 263.0 | 276.9 | 313.7 | 330.2 |
| 400 | 355.3 | 188.4 | 193.7 | 192.9 | 186.5 | 263.7 | 277.6 | 314.5 | 331.1 |
| 401 | 356.2 | 188.9 | 194.3 | 193.4 | 187.1 | 264.4 | 278.3 | 315.3 | 331.9 |
| 402 | 357.1 | 189.4 | 194.8 | 194.0 | 187.6 | 265.0 | 279.0 | 316.1 | 332.7 |
| 403 | 358.0 | 189.9 | 195.4 | 194.5 | 188.1 | 265.7 | 279.7 | 316.9 | 333.6 |
| 404 | 358.9 | 190.5 | 195.9 | 195.0 | 188.7 | 266.4 | 280.4 | 317.7 | 334.4 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar. and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 405 | 359.7 | 191.0 | 196.4 | 195.6 | 189.2 | 267.1 | 281.1 | 318.5 | 335.2 |
| 406 | 360.6 | 191.5 | 197.0 | 196.1 | 189.8 | 267.8 | 281.9 | 319.2 | 336.0 |
| 407 | 361.5 | 192.1 | 197.5 | 196.7 | 190.3 | 268.4 | 282.6 | 320.0 | 336.9 |
| 408 | 362.4 | 192.6 | 198.1 | 197.2 | 190.8 | 269.1 | 283.3 | 320.8 | 337.7 |
| 409 | 363.3 | 193.1 | 198.6 | 197.7 | 191.4 | 269.8 | 284.0 | 321.6 | 338.5 |
| 410 | 364.2 | 193.7 | 199.1 | 198.3 | 191.9 | 270.5 | 284.7 | 322.4 | 339.4 |
| 411 | 365.1 | 194.2 | 199.7 | 198.8 | 192.5 | 271.2 | 285.4 | 323.2 | 340.2 |
| 412 | 366.0 | 194.7 | 200.2 | 199.4 | 193.0 | 271.8 | 286.2 | 324.0 | 341.0 |
| 413 | 366.9 | 195.2 | 200.8 | 199.9 | 193.5 | 272.5 | 286.9 | 324.8 | 341.9 |
| 414 | 367.7 | 195.8 | 201.3 | 200.5 | 194.1 | 273.2 | 287.6 | 325.6 | 342.7 |
| 415 | 368.6 | 196.3 | 201.8 | 201.0 | 194.6 | 273.9 | 288.3 | 326.3 | 343.5 |
| 416 | 369.5 | 196.8 | 202.4 | 201.6 | 195.2 | 274.6 | 289.0 | 327.1 | 344.4 |
| 417 | 370.4 | 197.4 | 202.9 | 202.1 | 195.7 | 275.2 | 289.7 | 327.9 | 345.2 |
| 418 | 371.3 | 197.9 | 203.5 | 202.6 | 196.2 | 275.9 | 290.4 | 328.7 | 346.0 |
| 419 | 372.2 | 198.4 | 204.0 | 203.2 | 196.8 | 276.6 | 291.2 | 329.5 | 346.8 |
| 420 | 373.1 | 199.0 | 204.6 | 203.7 | 197.3 | 277.3 | 291.9 | 330.3 | 347.7 |
| 421 | 374.0 | 199.5 | 205.1 | 204.3 | 197.9 | 277.9 | 292.6 | 331.1 | 348.5 |
| 422 | 374.8 | 200.1 | 205.7 | 204.8 | 198.4 | 278.6 | 293.3 | 331.9 | 349.3 |
| 423 | 375.7 | 200.6 | 206.2 | 205.4 | 198.9 | 279.3 | 294.0 | 332.7 | 350.2 |
| 424 | 376.6 | 201.1 | 206.7 | 205.9 | 199.5 | 280.0 | 294.7 | 333.4 | 351.0 |
| 425 | 377.5 | 201.7 | 207.3 | 206.5 | 200.0 | 280.7 | 295.4 | 334.2 | 351.8 |
| 426 | 378.4 | 202.2 | 207.8 | 207.0 | 200.6 | 281.3 | 296.2 | 335.0 | 352.7 |
| 427 | 379.3 | 202.8 | 208.4 | 207.6 | 201.1 | 282.0 | 296.9 | 335.8 | 353.5 |
| 428 | 380.2 | 203.3 | 208.9 | 208.1 | 201.7 | 282.7 | 297.6 | 336.6 | 354.3 |
| 429 | 381.1 | 203.8 | 209.5 | 208.7 | 202.2 | 283.4 | 298.3 | 337.4 | 355.1 |
| 430 | 382.0 | 204.4 | 210.0 | 209.2 | 202.7 | 284.1 | 299.0 | 338.2 | 356.0 |
| 431 | 382.8 | 204.9 | 210.6 | 209.8 | 203.3 | 284.7 | 299.7 | 339.0 | 356.8 |
| 432 | 383.7 | 205.5 | 211.1 | 210.3 | 203.8 | 285.4 | 300.5 | 339.7 | 357.6 |
| 433 | 384.6 | 206.0 | 211.7 | 210.9 | 204.4 | 286.1 | 301.2 | 340.5 | 358.5 |
| 434 | 385.5 | 206.5 | 212.2 | 211.4 | 204.9 | 286.8 | 301.9 | 341.3 | 359.3 |
| 435 | 386.4 | 207.1 | 212.8 | 212.0 | 205.5 | 287.5 | 302.6 | 342.1 | 360.1 |
| 436 | 387.3 | 207.6 | 213.3 | 212.5 | 206.0 | 288.1 | 303.3 | 342.9 | 361.0 |
| 437 | 388.2 | 208.2 | 213.9 | 213.1 | 206.6 | 288.8 | 304.0 | 343.7 | 361.8 |
| 438 | 389.1 | 208.7 | 214.4 | 213.6 | 207.1 | 289.5 | 304.7 | 344.5 | 362.6 |
| 439 | 390.0 | 209.2 | 215.0 | 214.2 | 207.7 | 290.2 | 305.5 | 345.3 | 363.4 |
| 440 | 390.8 | 209.8 | 215.5 | 214.7 | 208.2 | 290.9 | 306.2 | 346.1 | 364.3 |
| 441 | 391.7 | 210.3 | 216.1 | 215.3 | 208.8 | 291.5 | 306.9 | 346.8 | 365.1 |
| 442 | 392.6 | 210.9 | 216.6 | 215.8 | 209.3 | 292.2 | 307.6 | 347.6 | 365.9 |
| 443 | 393.5 | 211.4 | 217.2 | 216.4 | 209.9 | 292.9 | 308.3 | 348.4 | 366.8 |
| 444 | 394.4 | 212.0 | 217.8 | 216.9 | 210.4 | 293.6 | 309.0 | 349.2 | 367.6 |

TABLE 19. (*Continued.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose) | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 445 | 395.3 | 212.5 | 218.3 | 217.5 | 211.0 | 294.2 | 309.7 | 350.0 | 368.4 |
| 446 | 396.2 | 213.1 | 218.9 | 218.0 | 211.5 | 294.9 | 310.5 | 350.8 | 369.3 |
| 447 | 397.1 | 213.6 | 219.4 | 218.6 | 212.1 | 295.6 | 311.2 | 351.6 | 370.1 |
| 448 | 397.9 | 214.1 | 220.0 | 219.1 | 212.6 | 296.3 | 311.9 | 352.4 | 370.9 |
| 449 | 398.8 | 214.7 | 220.5 | 219.7 | 213.2 | 297.0 | 312.6 | 353.2 | 371.7 |
| 450 | 399.7 | 215.2 | 221.1 | 220.2 | 213.7 | 297.6 | 313.3 | 353.9 | 372.6 |
| 451 | 400.6 | 215.8 | 221.6 | 220.8 | 214.3 | 298.3 | 314.0 | 354.7 | 373.4 |
| 452 | 401.5 | 216.3 | 222.2 | 221.4 | 214.8 | 299.0 | 314.7 | 355.5 | 374.2 |
| 453 | 402.4 | 216.9 | 222.8 | 221.9 | 215.4 | 299.7 | 315.5 | 356.3 | 375.1 |
| 454 | 403.3 | 217.4 | 223.3 | 222.5 | 215.9 | 300.4 | 316.2 | 357.1 | 375.9 |
| 455 | 404.2 | 218.0 | 223.9 | 223.0 | 216.5 | 301.1 | 316.9 | 357.9 | 376.7 |
| 456 | 405.1 | 218.5 | 224.4 | 223.6 | 217.0 | 301.7 | 317.6 | 358.7 | 377.6 |
| 457 | 405.9 | 219.1 | 225.0 | 224.1 | 217.6 | 302.4 | 318.3 | 359.5 | 378.4 |
| 458 | 406.8 | 219.6 | 225.5 | 224.7 | 218.1 | 303.1 | 319.0 | 360.3 | 379.2 |
| 459 | 407.7 | 220.2 | 226.1 | 225.3 | 218.7 | 303.8 | 319.8 | 361.0 | 380.0 |
| 460 | 408.6 | 220.7 | 226.7 | 225.8 | 219.2 | 304.5 | 320.5 | 361.8 | 380.9 |
| 461 | 409.5 | 221.3 | 227.2 | 226.4 | 219.8 | 305.1 | 321.2 | 362.6 | 381.7 |
| 462 | 410.4 | 221.8 | 227.8 | 226.9 | 220.3 | 305.8 | 321.9 | 363.4 | 382.5 |
| 463 | 411.3 | 222.4 | 228.3 | 227.5 | 220.9 | 306.5 | 322.6 | 364.2 | 383.4 |
| 464 | 412.2 | 222.9 | 228.9 | 228.1 | 221.4 | 307.2 | 323.4 | 365.0 | 384.2 |
| 465 | 413.0 | 223.5 | 229.5 | 228.6 | 222.0 | 307.9 | 324.1 | 365.8 | 385.0 |
| 466 | 413.9 | 224.0 | 230.0 | 229.2 | 222.5 | 308.6 | 324.8 | 366.6 | 385.9 |
| 467 | 414.8 | 224.6 | 230.6 | 229.7 | 223.1 | 309.2 | 325.5 | 367.3 | 386.7 |
| 468 | 415.7 | 225.1 | 231.2 | 230.3 | 223.7 | 309.9 | 326.2 | 368.1 | 387.5 |
| 469 | 416.6 | 225.7 | 231.7 | 230.9 | 224.2 | 310.6 | 326.9 | 368.9 | 388.3 |
| 470 | 417.5 | 226.2 | 232.3 | 231.4 | 224.8 | 311.3 | 327.7 | 369.7 | 389.2 |
| 471 | 418.4 | 226.8 | 232.8 | 232.0 | 225.3 | 312.0 | 328.4 | 370.5 | 390.0 |
| 472 | 419.3 | 227.4 | 233.4 | 232.5 | 225.9 | 312.6 | 329.1 | 371.3 | 390.8 |
| 473 | 420.2 | 227.9 | 234.0 | 233.1 | 226.4 | 313.3 | 329.8 | 372.1 | 391.7 |
| 474 | 421.0 | 228.5 | 234.5 | 233.7 | 227.0 | 314.0 | 330.5 | 372.9 | 392.5 |
| 475 | 421.9 | 229.0 | 235.1 | 234.2 | 227.6 | 314.7 | 331.3 | 373.7 | 393.3 |
| 476 | 422.8 | 229.6 | 235.7 | 234.8 | 228.1 | 315.4 | 332.0 | 374.4 | 394.2 |
| 477 | 423.7 | 230.1 | 236.2 | 235.4 | 228.7 | 316.1 | 332.7 | 375.2 | 395.0 |
| 478 | 424.6 | 230.7 | 236.8 | 235.9 | 229.2 | 316.7 | 333.4 | 376.0 | 395.8 |
| 479 | 425.5 | 231.3 | 237.4 | 236.5 | 229.8 | 317.4 | 334.1 | 376.8 | 396.6 |
| 480 | 426.4 | 231.8 | 237.9 | 237.1 | 230.3 | 318.1 | 334.8 | 377.6 | 397.5 |
| 481 | 427.3 | 232.4 | 238.5 | 237.6 | 230.9 | 318.8 | 335.6 | 378.4 | 398.3 |
| 482 | 428.1 | 232.9 | 239.1 | 238.2 | 231.5 | 319.5 | 336.3 | 379.2 | 399.1 |
| 483 | 429.0 | 233.5 | 239.6 | 238.8 | 232.0 | 320.1 | 337.0 | 380.0 | 400.0 |
| 484 | 429.9 | 234.1 | 240.2 | 239.3 | 232.6 | 320.8 | 337.7 | 380.7 | 400.8 |

TABLE 19. (*Concluded.*)

| Cuprous oxide ($Cu_2O$). | Copper (Cu). | Dextrose (*d*-glucose). | Invert sugar. | Invert sugar and sucrose. | | Lactose. | | Maltose. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.4 gram total sugar. | 2 grams total sugar. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. | $C_{12}H_{22}O_{11}$. | $C_{12}H_{22}O_{11}+H_2O$. |
| mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. |
| 485 | 430.8 | 234.6 | 240.8 | 239.9 | 233.2 | 321.5 | 338.4 | 381.5 | 401.6 |
| 486 | 431.7 | 235.2 | 241.4 | 240.5 | 233.7 | 322.2 | 339.1 | 382.3 | 402.4 |
| 487 | 432.6 | 235.7 | 241.9 | 241.0 | 234.3 | 322.9 | 339.9 | 383.1 | 403.3 |
| 488 | 433.5 | 236.3 | 242.5 | 241.6 | 234.8 | 323.6 | 340.6 | 383.9 | 404.1 |
| 489 | 434.4 | 236.9 | 243.1 | 242.2 | 235.4 | 324.2 | 341.3 | 384.7 | 404.9 |
| 490 | 435.3 | 237.4 | 243.6 | 242.7 | 236.0 | 324.9 | 342.0 | 385.5 | 405.8 |

## TABLE* 20.

### Bertrand's Table for Determining Invert Sugar, Glucose, Galactose, Maltose, and Lactose.

| Milligrams of sugar. | Milligrams of copper corresponding to | | | | |
|---|---|---|---|---|---|
| | Invert sugar. | Glucose. | Galactose. | Maltose. | Lactose. |
| **10** | 20.6 | 20.4 | 19.3 | 11.2 | 14.4 |
| 11 | 22.6 | 22.4 | 21.2 | 12.3 | 15.8 |
| 12 | 24.6 | 24.3 | 23.0 | 13.4 | 17.2 |
| 13 | 26.5 | 26.3 | 24.9 | 14.5 | 18.6 |
| 14 | 28.5 | 28.3 | 26.7 | 15.6 | 20.0 |
| 15 | 30.5 | 30.2 | 28.6 | 16.7 | 21.4 |
| 16 | 32.5 | 32.2 | 30.5 | 17.8 | 22.8 |
| 17 | 34.5 | 34.2 | 32.3 | 18.9 | 24.2 |
| 18 | 36.4 | 36.2 | 34.2 | 20.0 | 25.6 |
| 19 | 38.4 | 38.1 | 36.0 | 21 1 | 27.0 |
| **20** | 40.4 | 40.1 | 37.9 | 22 2 | 28 4 |
| 21 | 42.3 | 42.0 | 39.7 | 23.3 | 29.8 |
| 22 | 44.2 | 43.9 | 41.6 | 24.4 | 31.1 |
| 23 | 46.1 | 45.8 | 43.4 | 25.5 | 32.5 |
| 24 | 48.0 | 47.7 | 45.2 | 26.6 | 33.9 |
| 25 | 49.8 | 49.6 | 47.0 | 27.7 | 35.2 |
| 26 | 51.7 | 51.5 | 48.9 | 28.9 | 36.6 |
| 27 | 53.6 | 53.4 | 50.7 | 30.0 | 38.0 |
| 28 | 55.5 | 55.3 | 52.5 | 31.1 | 39.4 |
| 29 | 57.4 | 57.2 | 54.4 | 32.2 | 40.7 |
| **30** | 59.3 | 59.1 | 56.2 | 33.3 | 42.1 |
| 31 | 61.1 | 60.9 | 58.0 | 34.4 | 43.4 |
| 32 | 63.0 | 62.8 | 59.7 | 35.5 | 44.8 |
| 33 | 64.8 | 64.6 | 61.5 | 36.5 | 46.1 |
| 34 | 66.7 | 66.5 | 63.3 | 37.6 | 47.4 |
| 35 | 68.5 | 68.3 | 65.0 | 38.7 | 48.7 |
| 36 | 70.3 | 70.1 | 66.8 | 39.8 | 50.1 |
| 37 | 72.2 | 72.0 | 68.6 | 40.9 | 51.4 |
| 38 | 74.0 | 73.8 | 70.4 | 41.9 | 52.7 |
| 39 | 75.9 | 75.7 | 72.1 | 43.0 | 54.1 |
| **40** | 77.7 | 77.5 | 73.9 | 44.1 | 55.4 |
| 41 | 79.5 | 79.3 | 75.6 | 45.2 | 56.7 |
| 42 | 81.2 | 81.1 | 77.4 | 46.3 | 58.0 |
| 43 | 83.0 | 82.9 | 79.1 | 47.4 | 59.3 |
| 44 | 84.8 | 84.7 | 80.8 | 48.5 | 60.6 |
| 45 | 86.5 | 86.4 | 82.5 | 49.5 | 61.9 |
| 46 | 88.3 | 88.2 | 84.3 | 50.6 | 63.3 |
| 47 | 90.1 | 90.0 | 86.0 | 51.7 | 64.6 |
| 48 | 91.9 | 91.8 | 87.7 | 52.8 | 65.9 |
| 49 | 93.6 | 93.6 | 89.5 | 53.9 | 67.2 |
| **50** | 95.4 | 95.4 | 91.2 | 55.0 | 68.5 |
| 51 | 97.1 | 97.1 | 92.9 | 56.1 | 69.8 |
| 52 | 98.8 | 98.9 | 94.6 | 57.1 | 71.1 |
| 53 | 100.6 | 100.6 | 96.3 | 58.2 | 72.4 |
| 54 | 102.2 | 102.3 | 98.0 | 59.3 | 73.7 |
| 55 | 104.0 | 104.1 | 99.7 | 60.3 | 74.9 |
| 56 | 105.7 | 105.8 | 101.5 | 61.4 | 76.2 |
| 57 | 107.4 | 107.6 | 103.2 | 62.5 | 77.5 |
| 58 | 109.2 | 109.3 | 104.9 | 63.5 | 78.8 |
| 59 | 110.9 | 111.1 | 106.6 | 64.6 | 80.1 |

* *See* "Handbook," page 426.

TABLE 20. (*Concluded.*)

| Milligrams of sugar. | Milligrams of copper corresponding to | | | | |
|---|---|---|---|---|---|
| | Invert sugar. | Glucose. | Galactose. | Maltose. | Lactose. |
| **60** | 112.6 | 112.8 | 108.3 | 65.7 | 81.4 |
| 61 | 114.3 | 114.5 | 110.0 | 66.8 | 82.7 |
| 62 | 115.9 | 116.2 | 111.6 | 67.9 | 83.9 |
| 63 | 117.6 | 117.9 | 113.3 | 68.9 | 85.2 |
| 64 | 119.2 | 119.6 | 115.0 | 70.0 | 86.5 |
| 65 | 120.9 | 121.3 | 116.6 | 71.1 | 87.7 |
| 66 | 122.6 | 123.0 | 118.3 | 72.2 | 89.9 |
| 67 | 124.2 | 124.7 | 120.0 | 73.3 | 90.3 |
| 68 | 125.9 | 126.4 | 121.7 | 74.3 | 91.6 |
| 69 | 127.5 | 128.1 | 123.3 | 75.4 | 92.8 |
| **70** | 129.2 | 129.8 | 125.0 | 76.5 | 94.1 |
| 71 | 130.8 | 131.4 | 126.6 | 77.6 | 95.4 |
| 72 | 132.4 | 133.1 | 128.3 | 78.6 | 96.9 |
| 73 | 134.0 | 134.7 | 130.0 | 79.7 | 98.0 |
| 74 | 135.6 | 136.3 | 131.5 | 80.8 | 99.1 |
| 75 | 137.2 | 137.9 | 133.1 | 81.8 | 100.4 |
| 76 | 138.9 | 139.6 | 134.8 | 82.9 | 101.7 |
| 77 | 140.5 | 141.2 | 136.4 | 84.0 | 102.9 |
| 78 | 142.1 | 142.8 | 138.0 | 85.1 | 104.2 |
| 79 | 143.7 | 144.5 | 139.7 | 86.1 | 105.4 |
| **80** | 145.3 | 146.1 | 141.3 | 87.2 | 106.7 |
| 81 | 146.9 | 147.7 | 142.9 | 88.3 | 107.9 |
| 82 | 148.5 | 149.3 | 144.6 | 89.4 | 109.2 |
| 83 | 150.0 | 150.9 | 146.2 | 90.4 | 110.4 |
| 84 | 151.6 | 152.5 | 147.8 | 91.5 | 111 7 |
| 85 | 153.2 | 154.0 | 149.4 | 92.6 | 112.9 |
| 86 | 154.8 | 155.6 | 151.1 | 93.7 | 114.1 |
| 87 | 156.4 | 157.2 | 152.7 | 94.8 | 115.4 |
| 88 | 157.9 | 158.8 | 154.3 | 95.8 | 116.6 |
| 89 | 159.5 | 160.4 | 156.0 | 96.9 | 117.9 |
| **90** | 161.1 | 162.0 | 157.6 | 98.0 | 119.1 |
| 91 | 162.6 | 163.6 | 159.2 | 99.0 | 120.3 |
| 92 | 164.2 | 165.2 | 160.8 | 100.1 | 121.6 |
| 93 | 165.7 | 166.7 | 162.4 | 101.1 | 122.8 |
| 94 | 167.3 | 168.3 | 164.0 | 102.2 | 124.0 |
| 95 | 168.8 | 169.9 | 165.6 | 103.2 | 125.2 |
| 96 | 170.3 | 171.5 | 167.2 | 104.2 | 126.5 |
| 97 | 171.9 | 173.1 | 168.8 | 105.3 | 127.7 |
| 98 | 173.4 | 174.6 | 170.4 | 106.3 | 128.9 |
| 99 | 175.0 | 176.2 | 172.0 | 107.4 | 130.2 |
| **100** | 176.5 | 177.8 | 173.6 | 108.4 | 131.4 |

TABLE* 21.

HERZFELD'S TABLE FOR DETERMINING INVERT SUGAR IN RAW SUGARS (INVERT SUGAR NOT TO EXCEED 1.5%.)

| Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. |
|---|---|---|---|---|---|---|---|
| mgs. | per cent. | mgs. | per cent. | mgs. | per cent. | mgs. | per cent. |
| **50** | 0.050 | 101 | 0.305 | 152 | 0.574 | 203 | 0.863 |
| 51 | 0.054 | 102 | 0.310 | 153 | 0.580 | 204 | 0.869 |
| 52 | 0.058 | 103 | 0.315 | 154 | 0.586 | 205 | 0.874 |
| 53 | 0.062 | 104 | 0.320 | 155 | 0.592 | 206 | 0.880 |
| 54 | 0.066 | 105 | 0.325 | 156 | 0.598 | 207 | 0.885 |
| 55 | 0.070 | 106 | 0.330 | 157 | 0.604 | 208 | 0.891 |
| 56 | 0.074 | 107 | 0.335 | 158 | 0.609 | 209 | 0.896 |
| 57 | 0.078 | 108 | 0.340 | 159 | 0.615 | **210** | 0.902 |
| 58 | 0.082 | 109 | 0.346 | **160** | 0.621 | 211 | 0.907 |
| 59 | 0.086 | **110** | 0.351 | 161 | 0.627 | 212 | 0.913 |
| **60** | 0.090 | 111 | 0.356 | 162 | 0.633 | 213 | 0.918 |
| 61 | 0.094 | 112 | 0.361 | 163 | 0.639 | 214 | 0.924 |
| 62 | 0.098 | 113 | 0.366 | 164 | 0.645 | 215 | 0.929 |
| 63 | 0.103 | 114 | 0.371 | 165 | 0.651 | 216 | 0.935 |
| 64 | 0.108 | 115 | 0.376 | 166 | 0.657 | 217 | 0.940 |
| 65 | 0.113 | 116 | 0.381 | 167 | 0.663 | 218 | 0.946 |
| 66 | 0.118 | 117 | 0.386 | 168 | 0.669 | 219 | 0.951 |
| 67 | 0.123 | 118 | 0.392 | 169 | 0.675 | **220** | 0.957 |
| 68 | 0.128 | 119 | 0.397 | **170** | 0.680 | 221 | 0.962 |
| 69 | 0.133 | **120** | 0.402 | 171 | 0.686 | 222 | 0.968 |
| **70** | 0.138 | 121 | 0.407 | 172 | 0.692 | 223 | 0.973 |
| 71 | 0.143 | 122 | 0.412 | 173 | 0.698 | 224 | 0.979 |
| 72 | 0.148 | 123 | 0.417 | 174 | 0.704 | 225 | 0.984 |
| 73 | 0.152 | 124 | 0.423 | 175 | 0.709 | 226 | 0.990 |
| 74 | 0.157 | 125 | 0.428 | 176 | 0.715 | 227 | 0.996 |
| 75 | 0.162 | 126 | 0.433 | 177 | 0.720 | 228 | 1.001 |
| 76 | 0.167 | 127 | 0.438 | 178 | 0.726 | 229 | 1.007 |
| 77 | 0.172 | 128 | 0.443 | 179 | 0.731 | **230** | 1.013 |
| 78 | 0.177 | 129 | 0.448 | **180** | 0.737 | 231 | 1.018 |
| 79 | 0.182 | **130** | 0.453 | 181 | 0.742 | 232 | 1.024 |
| **80** | 0.187 | 131 | 0.458 | 182 | 0.748 | 233 | 1.030 |
| 81 | 0.192 | 132 | 0.463 | 183 | 0.753 | 234 | 1.036 |
| 82 | 0.197 | 133 | 0.468 | 184 | 0.759 | 235 | 1.041 |
| 83 | 0.202 | 134 | 0.473 | 185 | 0.764 | 236 | 1.047 |
| 84 | 0.208 | 135 | 0.478 | 186 | 0.770 | 237 | 1.053 |
| 85 | 0.213 | 136 | 0.483 | 187 | 0.775 | 238 | 1.058 |
| 86 | 0.219 | 137 | 0.488 | 188 | 0.781 | 239 | 1.064 |
| 87 | 0.225 | 138 | 0.493 | 189 | 0.786 | **240** | 1.070 |
| 88 | 0.231 | 139 | 0.498 | **190** | 0.792 | 241 | 1.076 |
| 89 | 0.236 | **140** | 0.503 | 191 | 0.797 | 242 | 1.081 |
| **90** | 0.242 | 141 | 0.509 | 192 | 0.803 | 243 | 1.087 |
| 91 | 0.248 | 142 | 0.515 | 193 | 0.808 | 244 | 1.093 |
| 92 | 0.254 | 143 | 0.521 | 194 | 0.814 | 245 | 1.099 |
| 93 | 0.260 | 144 | 0.527 | 195 | 0.819 | 246 | 1.104 |
| 94 | 0.265 | 145 | 0.533 | 196 | 0.825 | 247 | 1.110 |
| 95 | 0.271 | 146 | 0.538 | 197 | 0.830 | 248 | 1.116 |
| 96 | 0.277 | 147 | 0.544 | 198 | 0.836 | 249 | 1.122 |
| 97 | 0.283 | 148 | 0.550 | 199 | 0.841 | **250** | 1.127 |
| 98 | 0.288 | 149 | 0.556 | **200** | 0.847 | 251 | 1.133 |
| 99 | 0.294 | **150** | 0.562 | 201 | 0.852 | 252 | 1.139 |
| **100** | 0.300 | 151 | 0.568 | 202 | 0.858 | 253 | 1.144 |

* *See* " Handbook," page 428.

TABLE 21. (*Concluded.*)

| Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. | Copper. (Cu). | Invert sugar. |
|---|---|---|---|---|---|---|---|
| mgs. | per cent. | mgs. | per cent. | mgs. | per cent. | mgs. | per cent. |
| 254 | 1.150 | **270** | 1.242 | 286 | 1.334 | 302 | 1.425 |
| 255 | 1.156 | 271 | 1.248 | 287 | 1.339 | 303 | 1.431 |
| 256 | 1.162 | 272 | 1.253 | 288 | 1.345 | 304 | 1.437 |
| 257 | 1.167 | 273 | 1.259 | 289 | 1.351 | 305 | 1.443 |
| 258 | 1.173 | 274 | 1.265 | **290** | 1.357 | 306 | 1.448 |
| 259 | 1.179 | 275 | 1.271 | 291 | 1.362 | 307 | 1.454 |
| **260** | 1.185 | 276 | 1.276 | 292 | 1.368 | 308 | 1.460 |
| 261 | 1.190 | 277 | 1.282 | 293 | 1.374 | 309 | 1.466 |
| 262 | 1.196 | 278 | 1.288 | 294 | 1.380 | **310** | 1.471 |
| 263 | 1.202 | 279 | 1.294 | 295 | 1.385 | 311 | 1.477 |
| 264 | 1.207 | **280** | 1.299 | 296 | 1.391 | 312 | 1.483 |
| 265 | 1.213 | 281 | 1.305 | 297 | 1.397 | 313 | 1.489 |
| 266 | 1.219 | 282 | 1.311 | 298 | 1.403 | 314 | 1.494 |
| 267 | 1.225 | 283 | 1.317 | 299 | 1.408 | 315 | 1.500 |
| 268 | 1.231 | 284 | 1.322 | **300** | 1.414 | | |
| 269 | 1.236 | 285 | 1.328 | 301 | 1.420 | | |

TABLE * 22.

KRÖBER'S TABLE FOR DETERMINING PENTOSES AND PENTOSANS.

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.030 | 0.0182 | 0.0391 | 0.0344 | 0.0324 | 0.0285 | 0.0358 | 0.0315 |
| .031 | .0188 | .0402 | .0354 | .0333 | .0293 | .0368 | .0324 |
| .032 | .0193 | .0413 | .0363 | .0342 | .0301 | .0378 | .0333 |
| .033 | .0198 | .0424 | .0373 | .0352 | .0309 | .0388 | .0341 |
| .034 | .0203 | .0435 | .0383 | .0361 | .0317 | .0398 | .0350 |
| .035 | .0209 | .0446 | .0393 | .0370 | .0326 | .0408 | .0359 |
| .036 | .0214 | .0457 | .0402 | .0379 | .0334 | .0418 | .0368 |
| .037 | .0219 | .0468 | .0412 | .0388 | .0342 | .0428 | .0377 |
| .038 | .0224 | .0479 | .0422 | .0398 | .0350 | .0439 | .0386 |
| .039 | .0229 | .0490 | .0431 | .0407 | .0358 | .0449 | .0395 |
| .040 | .0235 | .0501 | .0441 | .0416 | .0366 | .0459 | .0404 |
| .041 | .0240 | .0512 | .0451 | .0425 | .0374 | .0469 | .0413 |
| .042 | .0245 | .0523 | .0460 | .0434 | .0382 | .0479 | .0422 |
| .043 | .0250 | .0534 | .0470 | .0443 | .0390 | .0489 | .0431 |
| .044 | .0255 | .0545 | .0480 | .0452 | .0398 | .0499 | .0440 |
| .045 | .0260 | .0556 | .0490 | .0462 | .0406 | .0509 | .0448 |
| .046 | .0266 | .0567 | .0499 | .0471 | .0414 | .0519 | .0457 |
| .047 | .0271 | .0578 | .0509 | .0480 | .0422 | .0529 | .0466 |
| .048 | .0276 | .0589 | .0519 | .0489 | .0430 | .0539 | .0475 |
| .049 | .0281 | .0600 | .0528 | .0498 | .0438 | .0549 | .0484 |
| .050 | .0286 | .0611 | .0538 | .0507 | .0446 | .0559 | .0492 |
| .051 | .0292 | .0622 | .0548 | .0516 | .0454 | .0569 | .0501 |
| .052 | .0297 | .0633 | .0557 | .0525 | .0462 | .0579 | .0510 |
| .053 | .0302 | .0644 | .0567 | .0534 | .0470 | .0589 | .0519 |
| .054 | .0307 | .0655 | .0576 | .0543 | .0478 | .0599 | .0528 |
| .055 | .0312 | .0666 | .0586 | .0553 | .0486 | .0610 | .0537 |
| .056 | .0318 | .0677 | .0596 | .0562 | .0494 | .0620 | .0546 |
| .057 | .0323 | .0688 | .0605 | .0571 | .0502 | .0630 | .0555 |
| .058 | .0328 | .0699 | .0615 | .0580 | .0510 | .0640 | .0564 |
| .059 | .0333 | .0710 | .0624 | .0589 | .0518 | .0650 | .0573 |
| .060 | .0338 | .0721 | .0634 | .0598 | .0526 | .0660 | .0581 |
| .061 | .0344 | .0732 | .0644 | .0607 | .0534 | .0670 | .0590 |
| .062 | .0349 | .0743 | .0653 | .0616 | .0542 | .0680 | .0599 |
| .063 | .0354 | .0754 | .0663 | .0626 | .0550 | .0690 | .0608 |
| .064 | .0359 | .0765 | .0673 | .0635 | .0558 | .0700 | .0617 |
| .065 | .0364 | .0776 | .0683 | .0644 | .0567 | .0710 | .0625 |
| .066 | .0370 | 0787 | .0692 | .0653 | .0575 | .0720 | .0634 |
| .067 | .0375 | .0798 | .0702 | .0662 | .0583 | .0730 | .0643 |
| .068 | .0380 | .0809 | .0712 | .0672 | .0591 | .0741 | .0652 |
| .069 | .0385 | .0820 | .0721 | .0681 | .0599 | .0751 | .0661 |
| .070 | .0390 | .0831 | .0731 | .0690 | .0607 | .0761 | .0670 |
| .071 | .0396 | .0842 | .0741 | .0699 | .0615 | .0771 | .0679 |
| .072 | .0401 | .0853 | .0750 | .0708 | .0623 | .0781 | .0688 |
| .073 | .0406 | .0864 | .0760 | .0717 | .0631 | .0791 | .0697 |
| .074 | .0411 | .0875 | .0770 | .0726 | .0639 | .0801 | .0706 |

* *See* "Handbook," page 450.

TABLE 22. (*Continued.*)

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.075 | 0.0416 | 0.0886 | 0.0780 | 0.0736 | 0.0647 | 0.0811 | 0.0714 |
| .076 | .0422 | .0897 | .0789 | .0745 | .0655 | .0821 | .0722 |
| .077 | .0427 | .0908 | .0799 | .0754 | .0663 | .0831 | .0731 |
| .078 | .0432 | .0919 | .0809 | .0763 | .0671 | .0841 | .0740 |
| .079 | .0437 | .0930 | .0818 | .0772 | .0679 | .0851 | .0749 |
| .080 | .0442 | .0941 | .0828 | .0781 | .0687 | .0861 | .0758 |
| .081 | .0448 | .0952 | .0838 | .0790 | .0695 | .0871 | .0767 |
| .082 | .0453 | .0963 | .0847 | .0799 | .0703 | .0881 | .0776 |
| .083 | .0458 | .0974 | .0857 | .0808 | .0711 | .0891 | .0785 |
| .084 | .0463 | .0985 | .0867 | .0817 | .0719 | .0901 | .0794 |
| .085 | .0468 | .0996 | .0877 | .0827 | .0727 | .0912 | .0803 |
| .086 | .0474 | .1007 | .0886 | .0836 | .0735 | .0922 | .0812 |
| .087 | .0479 | .1018 | .0896 | .0845 | .0743 | .0932 | .0821 |
| .088 | .0484 | .1029 | .0906 | .0854 | .0751 | .0942 | .0830 |
| .089 | .0489 | .1040 | .0915 | .0863 | .0759 | .0952 | .0838 |
| .090 | .0494 | .1051 | .0925 | .0872 | .0767 | .0962 | .0847 |
| .091 | .0499 | .1062 | .0935 | .0881 | .0775 | .0972 | .0856 |
| .092 | .0505 | .1073 | .0944 | .0890 | .0783 | .0982 | .0865 |
| .093 | .0510 | .1084 | .0954 | .0900 | .0791 | .0992 | .0874 |
| .094 | .0515 | .1095 | .0964 | .0909 | .0800 | .1002 | .0883 |
| .095 | .0520 | .1106 | .0974 | .0918 | .0808 | .1012 | .0891 |
| .096 | .0525 | .1117 | .0983 | .0927 | .0816 | .1022 | .0899 |
| .097 | .0531 | .1128 | .0993 | .0936 | .0824 | .1032 | .0908 |
| .098 | .0536 | .1139 | .1003 | .0946 | .0832 | .1043 | .0917 |
| .099 | .0541 | .1150 | .1012 | .0955 | .0840 | .1053 | .0926 |
| .100 | .0546 | .1161 | .1022 | .0964 | .0848 | .1063 | .0935 |
| .101 | .0551 | .1171 | .1032 | .0973 | .0856 | .1073 | .0944 |
| .102 | .0557 | .1182 | .1041 | .0982 | .0864 | .1083 | .0953 |
| .103 | .0562 | .1193 | .1051 | .0991 | .0872 | .1093 | .0962 |
| .104 | .0567 | .1204 | .1060 | .1000 | .0880 | .1103 | .0971 |
| .105 | .0572 | .1215 | .1070 | .1010 | .0888 | .1113 | .0979 |
| .106 | .0577 | .1226 | .1080 | .1019 | .0896 | .1123 | .0988 |
| .107 | .0582 | .1237 | .1089 | .1028 | .0904 | .1133 | .0997 |
| .108 | .0588 | .1248 | .1099 | .1037 | .0912 | .1143 | .1006 |
| .109 | .0593 | .1259 | .1108 | .1046 | .0920 | .1153 | .1015 |
| .110 | .0598 | .1270 | .1118 | .1055 | .0928 | .1163 | .1023 |
| .111 | .0603 | .1281 | .1128 | .1064 | .0936 | .1173 | .1032 |
| .112 | .0608 | .1292 | .1137 | .1073 | .0944 | .1183 | .1041 |
| .113 | .0614 | .1303 | .1147 | .1082 | .0952 | .1193 | .1050 |
| .114 | .0619 | .1314 | .1156 | .1091 | .0960 | .1203 | .1059 |
| .115 | .0624 | .1325 | .1166 | .1101 | .0968 | .1213 | .1067 |
| .116 | .0629 | .1336 | .1176 | .1110 | .0976 | .1223 | .1076 |
| .117 | .0634 | .1347 | .1185 | .1119 | .0984 | .1233 | .1085 |
| .118 | .0640 | .1358 | .1195 | .1128 | .0992 | .1243 | .1094 |
| .119 | .0645 | .1369 | .1204 | .1137 | .1000 | .1253 | .1103 |

TABLE 22. (*Continued.*)

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.120 | 0.0650 | 0.1380 | 0.1214 | 0.1146 | 0.1008 | 0.1263 | 0.1111 |
| .121 | .0655 | .1391 | .1224 | .1155 | .1016 | .1273 | .1120 |
| .122 | .0660 | .1402 | .1233 | .1164 | .1024 | .1283 | .1129 |
| .123 | .0665 | .1413 | .1243 | .1173 | .1032 | .1293 | .1138 |
| .124 | .0671 | .1424 | .1253 | .1182 | .1040 | .1303 | .1147 |
| .125 | .0676 | .1435 | .1263 | .1192 | .1049 | .1314 | .1156 |
| .126 | .0681 | .1446 | 1272 | .1201 | .1057 | .1324 | .1165 |
| .127 | .0686 | .1457 | .1282 | .1210 | .1065 | .1334 | .1174 |
| .128 | .0691 | .1468 | .1292 | .1219 | .1073 | .1344 | .1183 |
| .129 | .0697 | .1479 | .1301 | .1228 | .1081 | .1354 | .1192 |
| .130 | .0702 | .1490 | .1311 | .1237 | .1089 | .1364 | .1201 |
| .131 | .0707 | .1501 | .1321 | .1246 | .1097 | .1374 | .1210 |
| .132 | .0712 | .1512 | .1330 | .1255 | .1105 | .1384 | .1219 |
| .133 | .0717 | .1523 | .1340 | .1264 | .1113 | .1394 | .1227 |
| .134 | .0723 | .1534 | .1350 | .1273 | .1121 | .1404 | .1236 |
| .135 | .0728 | .1545 | .1360 | .1283 | .1129 | .1414 | .1244 |
| .136 | .0733 | .1556 | .1369 | .1292 | .1137 | .1424 | .1253 |
| .137 | .0738 | .1567 | .1379 | .1301 | .1145 | .1434 | .1262 |
| .138 | .0743 | .1578 | .1389 | .1310 | .1153 | .1444 | .1271 |
| .139 | .0748 | .1589 | .1398 | .1319 | .1161 | .1454 | .1280 |
| .140 | .0754 | .1600 | .1408 | .1328 | .1169 | .1464 | .1288 |
| .141 | .0759 | .1611 | .1418 | .1337 | .1177 | .1474 | .1297 |
| .142 | .0764 | .1622 | .1427 | .1346 | .1185 | .1484 | .1306 |
| .143 | .0769 | .1633 | .1437 | .1355 | .1193 | .1494 | .1315 |
| .144 | .0774 | .1644 | .1447 | .1364 | .1201 | .1504 | .1324 |
| .145 | .0780 | .1655 | .1457 | .1374 | .1209 | .1515 | .1333 |
| .146 | .0785 | .1666 | .1466 | .1383 | .1217 | .1525 | .1342 |
| .147 | .0790 | .1677 | .1476 | .1392 | .1225 | .1535 | .1351 |
| .148 | .0795 | .1688 | .1486 | .1401 | .1233 | .1545 | .1360 |
| .149 | .0800 | .1699 | .1495 | .1410 | .1241 | .1555 | .1369 |
| .150 | .0805 | .1710 | .1505 | .1419 | .1249 | .1565 | .1377 |
| .151 | .0811 | .1721 | .1515 | .1428 | .1257 | .1575 | .1386 |
| .152 | .0816 | .1732 | .1524 | .1437 | .1265 | .1585 | .1395 |
| .153 | .0821 | .1743 | .1534 | .1446 | .1273 | .1595 | .1404 |
| .154 | .0826 | .1754 | .1544 | .1455 | .1281 | .1605 | .1413 |
| .155 | .0831 | .1765 | .1554 | .1465 | .1289 | .1615 | .1421 |
| .156 | .0837 | .1776 | .1563 | .1474 | .1297 | .1625 | .1430 |
| .157 | .0842 | .1787 | .1573 | .1483 | .1305 | .1635 | .1439 |
| .158 | .0847 | .1798 | .1583 | .1492 | .1313 | .1645 | .1448 |
| .159 | .0852 | .1809 | .1592 | .1501 | .1321 | .1655 | .1457 |
| .160 | .0857 | .1820 | .1602 | .1510 | .1329 | .1665 | .1465 |
| .161 | .0863 | .1831 | .1612 | .1519 | .1337 | .1675 | .1474 |
| .162 | .0868 | .1842 | .1621 | .1528 | .1345 | .1685 | .1483 |
| .163 | .0873 | .1853 | .1631 | .1537 | .1353 | .1695 | .1492 |
| .164 | .0878 | .1864 | .1640 | .1546 | .1361 | .1705 | .1501 |

TABLE 22. (*Continued.*)

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.165 | 0.0883 | 0.1875 | 0.1650 | 0.1556 | 0.1369 | 0.1716 | 0.1510 |
| .166 | .0888 | .1886 | .1660 | .1565 | .1377 | .1726 | .1519 |
| .167 | .0894 | .1897 | .1669 | .1574 | .1385 | .1736 | .1528 |
| .168 | .0899 | .1908 | .1679 | .1583 | .1393 | .1746 | .1537 |
| .169 | .0904 | .1919 | .1688 | .1592 | .1401 | .1756 | .1546 |
| .170 | .0909 | .1930 | .1698 | .1601 | .1409 | .1766 | .1554 |
| .171 | .0914 | .1941 | .1708 | .1610 | .1417 | .1776 | .1563 |
| .172 | .0920 | .1952 | .1717 | .1619 | .1425 | .1786 | .1572 |
| .173 | .0925 | .1963 | .1727 | .1628 | .1433 | .1796 | .1581 |
| .174 | .0930 | .1974 | .1736 | .1637 | .1441 | .1806 | .1590 |
| .175 | .0935 | .1985 | .1746 | .1647 | .1449 | .1816 | .1598 |
| .176 | .0940 | .1996 | .1756 | .1656 | .1457 | .1826 | .1607 |
| .177 | .0946 | .2007 | .1765 | .1665 | .1465 | .1836 | .1616 |
| .178 | .0951 | .2018 | .1775 | .1674 | .1473 | .1846 | .1625 |
| .179 | .0956 | .2029 | .1784 | .1683 | .1481 | .1856 | .1634 |
| .180 | .0961 | .2039 | .1794 | .1692 | .1489 | .1866 | .1642 |
| .181 | .0966 | .2050 | .1804 | .1701 | .1497 | .1876 | .1651 |
| .182 | .0971 | .2061 | .1813 | .1710 | .1505 | .1886 | .1660 |
| .183 | .0977 | .2072 | .1823 | .1719 | .1513 | .1896 | .1669 |
| .184 | .0982 | .2082 | .1832 | .1728 | .1521 | .1906 | .1678 |
| .185 | .0987 | .2093 | .1842 | .1738 | .1529 | .1916 | .1686 |
| .186 | .0992 | .2104 | .1851 | .1747 | .1537 | .1926 | .1695 |
| .187 | .0997 | .2115 | .1861 | .1756 | .1545 | .1936 | .1704 |
| .188 | .1003 | .2126 | .1870 | .1765 | .1553 | .1946 | .1712 |
| .189 | .1008 | .2136 | .1880 | .1774 | .1561 | .1955 | .1721 |
| .190 | .1013 | .2147 | .1889 | .1783 | .1569 | .1965 | .1729 |
| .191 | .1018 | .2158 | .1899 | .1792 | .1577 | .1975 | .1738 |
| .192 | .1023 | .2168 | .1908 | .1801 | .1585 | .1985 | .1747 |
| .193 | .1028 | .2179 | .1918 | .1810 | .1593 | .1995 | .1756 |
| .194 | .1034 | .2190 | .1927 | .1819 | .1601 | .2005 | .1764 |
| .195 | .1039 | .2201 | .1937 | .1829 | .1609 | .2015 | .1773 |
| .196 | .1044 | .2212 | .1946 | .1838 | .1617 | .2025 | .1782 |
| .197 | .1049 | .2222 | .1956 | .1847 | .1625 | .2035 | .1791 |
| .198 | .1054 | .2233 | .1965 | .1856 | .1633 | .2045 | .1800 |
| .199 | .1059 | .2244 | .1975 | .1865 | .1641 | .2055 | .1808 |
| .200 | .1065 | .2255 | .1984 | .1874 | .1649 | .2065 | .1817 |
| .201 | .1070 | .2266 | .1994 | .1883 | .1657 | .2075 | .1826 |
| .202 | .1075 | .2276 | .2003 | .1892 | .1665 | .2085 | .1835 |
| .203 | .1080 | .2287 | .2013 | .1901 | .1673 | .2095 | .1844 |
| .204 | .1085 | .2298 | .2022 | .1910 | .1681 | .2105 | .1853 |
| .205 | .1090 | .2309 | .2032 | .1920 | .1689 | .2115 | .1861 |
| .206 | .1096 | .2320 | .2041 | .1929 | .1697 | .2125 | .1869 |
| .207 | .1101 | .2330 | .2051 | .1938 | .1705 | .2134 | .1878 |
| .208 | .1106 | .2341 | .2060 | .1947 | .1713 | .2144 | .1887 |
| .209 | .1111 | .2352 | .2069 | .1956 | .1721 | .2154 | .1896 |

TABLE 22. (*Continued.*)

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.210 | 0.1116 | 0.2363 | 0.2079 | 0.1965 | 0.1729 | 0.2164 | 0.1904 |
| .211 | .1121 | .2374 | .2089 | .1975 | .1737 | .2174 | .1913 |
| .212 | .1127 | .2384 | .2098 | .1984 | .1745 | .2184 | .1922 |
| .213 | .1132 | .2395 | .2108 | .1993 | .1753 | .2194 | .1931 |
| .214 | .1137 | .2406 | .2117 | .2002 | .1761 | .2204 | .1940 |
| .215 | .1142 | .2417 | .2127 | .2011 | .1770 | .2214 | .1948 |
| .216 | .1147 | .2428 | .2136 | .2020 | .1778 | .2224 | .1957 |
| .217 | .1152 | .2438 | .2146 | .2029 | .1786 | .2234 | .1966 |
| .218 | .1158 | .2449 | .2155 | .2038 | .1794 | .2244 | .1974 |
| .219 | .1163 | .2460 | .2165 | .2047 | .1802 | .2254 | .1983 |
| .220 | .1168 | .2471 | .2174 | .2057 | .1810 | .2264 | .1992 |
| .221 | .1173 | .2482 | .2184 | .2066 | .1818 | .2274 | .2001 |
| .222 | .1178 | .2492 | .2193 | .2075 | .1826 | .2284 | .2010 |
| .223 | .1183 | .2503 | .2203 | .2084 | .1834 | .2294 | .2019 |
| .224 | .1189 | .2514 | .2212 | .2093 | .1842 | .2304 | .2028 |
| .225 | .1194 | .2525 | .2222 | .2102 | .1850 | .2314 | .2037 |
| .226 | .1199 | .2536 | .2232 | .2111 | .1858 | .2324 | .2046 |
| .227 | .1204 | .2546 | .2241 | .2121 | .1866 | .2334 | .2054 |
| .228 | .1209 | .2557 | .2251 | .2130 | .1874 | .2344 | .2063 |
| .229 | .1214 | .2568 | .2260 | .2139 | .1882 | .2354 | .2072 |
| .230 | .1220 | .2579 | .2270 | .2148 | .1890 | .2364 | .2081 |
| .231 | .1225 | .2590 | .2280 | .2157 | .1898 | .2374 | .2089 |
| .232 | .1230 | .2600 | .2289 | .2166 | .1906 | .2383 | .2097 |
| .233 | .1235 | .2611 | .2299 | .2175 | .1914 | .2393 | .2106 |
| .234 | .1240 | .2622 | .2308 | .2184 | .1922 | .2403 | .2115 |
| .235 | .1245 | .2633 | .2318 | .2193 | .1930 | .2413 | .2124 |
| .236 | .1251 | .2644 | .2327 | .2202 | .1938 | .2423 | .2132 |
| .237 | .1256 | .2654 | .2337 | .2211 | .1946 | .2433 | .2141 |
| .238 | .1261 | .2665 | .2346 | .2220 | .1954 | .2443 | .2150 |
| .239 | .1266 | .2676 | .2356 | .2229 | .1962 | .2453 | .2159 |
| .240 | .1271 | .2687 | .2365 | .2239 | .1970 | .2463 | .2168 |
| .241 | .1276 | .2698 | .2375 | .2248 | .1978 | .2473 | .2176 |
| .242 | .1281 | .2708 | .2384 | .2257 | .1986 | .2483 | .2185 |
| .243 | .1287 | .2719 | .2394 | .2266 | .1994 | .2493 | .2194 |
| .244 | .1292 | .2730 | .2403 | .2275 | .2002 | .2503 | .2203 |
| .245 | .1297 | .2741 | .2413 | .2284 | .2010 | .2513 | .2212 |
| .246 | .1302 | .2752 | .2422 | .2293 | .2018 | .2523 | .2220 |
| .247 | .1307 | .2762 | .2432 | .2302 | .2026 | .2533 | .2229 |
| .248 | .1312 | .2773 | .2441 | .2311 | .2034 | .2543 | .2238 |
| .249 | .1318 | .2784 | .2451 | .2320 | .2042 | .2553 | .2247 |
| .250 | .1323 | .2795 | .2460 | .2330 | .2050 | .2563 | .2256 |
| .251 | .1328 | .2806 | .2470 | .2339 | .2058 | .2573 | .2264 |
| .252 | .1333 | .2816 | .2479 | .2348 | .2066 | .2582 | .2272 |
| .253 | .1338 | .2827 | .2489 | .2357 | .2074 | .2592 | .2281 |
| .254 | .1343 | .2838 | .2498 | .2366 | .2082 | .2602 | .2290 |

TABLE 22. (*Concluded.*)

| Furfural phloroglucide. | Furfural. | Arabinose. | Araban. | Xylose. | Xylan. | Pentose. | Pentosan. |
|---|---|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. | grams. | grams. |
| 0.255 | 0.1349 | 0.2849 | 0.2508 | 0.2375 | 0.2090 | 0.2612 | 0.2299 |
| .256 | 1354 | .2860 | .2517 | .2384 | .2098 | .2622 | .2307 |
| .257 | .1359 | .2870 | .2526 | .2393 | .2106 | .2632 | .2316 |
| .258 | .1364 | .2881 | .2536 | .2402 | .2114 | .2642 | .2325 |
| .259 | .1369 | .2892 | .2545 | .2411 | .2122 | .2652 | .2334 |
| .260 | .1374 | .2903 | .2555 | .2420 | .2130 | .2662 | 2342 |
| .261 | .1380 | .2914 | .2565 | .2429 | .2138 | .2672 | .2351 |
| .262 | .1385 | .2924 | .2574 | .2438 | .2146 | .2681 | .2359 |
| .263 | .1390 | .2935 | .2584 | .2447 | .2154 | .2691 | .2368 |
| .264 | .1395 | .2946 | .2593 | .2456 | .2162 | .2701 | .2377 |
| .265 | .1400 | .2957 | .2603 | .2465 | .2170 | .2711 | .2385 |
| .266 | .1405 | .2968 | .2612 | .2474 | .2178 | .2721 | .2394 |
| .267 | .1411 | .2978 | .2622 | .2483 | .2186 | .2731 | .2403 |
| .268 | .1416 | .2989 | .2631 | .2492 | .2194 | .2741 | .2412 |
| .269 | .1421 | .3000 | .2641 | .2502 | .2202 | .2751 | .2421 |
| .270 | .1426 | .3011 | .2650 | .2511 | .2210 | .2761 | .2429 |
| .271 | .1431 | .3022 | .2660 | .2520 | .2218 | .2771 | .2438 |
| .272 | .1436 | .3032 | .2669 | .2529 | .2226 | .2781 | .2447 |
| .273 | .1442 | .3043 | .2679 | .2538 | .2234 | .2791 | .2456 |
| .274 | .1447 | .3054 | .2688 | .2547 | .2242 | .2801 | .2465 |
| .275 | .1452 | .3065 | .2698 | .2556 | .2250 | .2811 | .2473 |
| .276 | .1457 | .3076 | .2707 | .2565 | .2258 | .2821 | .2482 |
| .277 | .1462 | .3086 | .2717 | .2574 | .2266 | .2830 | .2490 |
| .278 | .1467 | .3097 | .2726 | .2583 | .2274 | .2840 | .2499 |
| .279 | .1473 | .3108 | .2736 | .2592 | .2282 | .2850 | .2508 |
| .280 | .1478 | .3119 | .2745 | .2602 | .2290 | .2861 | .2517 |
| .281 | .1483 | .3130 | .2755 | .2611 | .2298 | .2871 | .2526 |
| .282 | .1488 | .3140 | .2764 | .2620 | .2306 | .2880 | .2534 |
| .283 | .1493 | .3151 | .2774 | .2629 | .2314 | .2890 | .2543 |
| .284 | .1498 | .3162 | .2783 | .2638 | .2322 | .2900 | .2552 |
| .285 | .1504 | .3173 | .2793 | .2647 | .2330 | .2910 | .2561 |
| .286 | .1509 | .3184 | .2802 | .2656 | .2338 | .2920 | .2570 |
| .287 | .1514 | .3194 | .2812 | .2665 | .2346 | .2930 | .2578 |
| .288 | .1519 | .3205 | .2821 | .2674 | .2354 | .2940 | .2587 |
| .289 | .1524 | .3216 | .2831 | .2683 | .2362 | .2950 | .2596 |
| .290 | .1529 | .3227 | .2840 | .2693 | .2370 | .2960 | .2605 |
| .291 | .1535 | .3238 | .2850 | .2702 | .2378 | .2970 | .2614 |
| .292 | .1540 | .3248 | .2859 | .2711 | .2386 | .2980 | .2622 |
| .293 | .1545 | .3259 | .2868 | .2720 | .2394 | .2990 | .2631 |
| .294 | .1550 | .3270 | .2878 | .2729 | .2402 | .3000 | .2640 |
| .295 | .1555 | .3281 | .2887 | .2738 | .2410 | .3010 | .2649 |
| .296 | .1560 | .3292 | .2897 | .2747 | .2418 | .3020 | .2658 |
| .297 | .1566 | .3302 | .2906 | .2756 | .2426 | .3030 | .2666 |
| .298 | .1571 | .3313 | .2916 | .2765 | .2434 | .3040 | .2675 |
| .299 | .1576 | .3324 | .2925 | .2774 | .2442 | .3050 | .2684 |
| .300 | .1581 | .3335 | .2935 | .2784 | .2450 | .3060 | .2693 |

## TABLE * 23.

### Tollens, Ellet, and Mayer's Table for Determining Methylpentoses and Methylpentosans.

| Methylfurfural phloroglucide. | Fucose. | Fucosan (fucose ×0.89). | Rhamnose. | Rhamnosan (rhamnose ×0.8). | Methylpentosan (average of fucosan and rhamnosan). |
|---|---|---|---|---|---|
| grams. | grams. | grams. | grams. | grams. | grams. |
| 0.010 | 0.0260 | 0.0231 | 0.0266 | 0.0213 | 0.0222 |
| 0.011 | 0.0284 | 0.0253 | 0.0279 | 0.0223 | 0.0238 |
| 0.012 | 0.0307 | 0.0274 | 0.0295 | 0.0236 | 0.0255 |
| 0.013 | 0.0331 | 0.0295 | 0.0311 | 0.0249 | 0.0272 |
| 0.014 | 0.0354 | 0.0315 | 0.0327 | 0.0262 | 0.0288 |
| 0.015 | 0.0377 | 0.0336 | 0.0343 | 0.0274 | 0.0305 |
| 0.016 | 0.0400 | 0.0356 | 0.0359 | 0.0287 | 0.0321 |
| 0.017 | 0.0423 | 0.0376 | 0.0375 | 0.0300 | 0.0338 |
| 0.018 | 0.0445 | 0.0396 | 0.0391 | 0.0313 | 0.0354 |
| 0.019 | 0.0467 | 0.0416 | 0.0407 | 0.0326 | 0.0371 |
| 0.020 | 0.0489 | 0.0435 | 0.0423 | 0.0338 | 0.0386 |
| 0.021 | 0.0510 | 0.0454 | 0.0438 | 0.0350 | 0.0402 |
| 0.022 | 0.0532 | 0.0473 | 0.0454 | 0.0363 | 0.0418 |
| 0.023 | 0.0553 | 0.0492 | 0.0469 | 0.0375 | 0.0433 |
| 0.024 | 0.0574 | 0.0511 | 0.0485 | 0.0388 | 0.0449 |
| 0.025 | 0.0594 | 0.0529 | 0.0500 | 0.0400 | 0.0462 |
| 0.026 | 0.0614 | 0.0547 | 0.0516 | 0.0413 | 0.0480 |
| 0.027 | 0.0634 | 0.0565 | 0.0531 | 0.0425 | 0.0495 |
| 0.028 | 0.0654 | 0.0583 | 0.0547 | 0.0438 | 0.0510 |
| 0.029 | 0.0674 | 0.0600 | 0.0562 | 0.0450 | 0.0525 |
| 0.030 | 0.0693 | 0.0617 | 0.0578 | 0.0462 | 0.0539 |
| 0.031 | 0.0712 | 0.0634 | 0.0593 | 0.0474 | 0.0554 |
| 0.032 | 0.0731 | 0.0651 | 0.0609 | 0.0487 | 0.0569 |
| 0.033 | 0.0750 | 0.0668 | 0.0624 | 0.0499 | 0.0584 |
| 0.034 | 0.0768 | 0.0684 | 0.0639 | 0.0511 | 0.0598 |
| 0.035 | 0.0786 | 0.0700 | 0.0655 | 0.0524 | 0.0612 |
| 0.036 | 0.0804 | 0.0716 | 0.0670 | 0.0536 | 0.0626 |
| 0.037 | 0.0822 | 0.0732 | 0.0685 | 0.0548 | 0.0640 |
| 0.038 | 0.0839 | 0.0747 | 0.0700 | 0.0560 | 0.0654 |
| 0.039 | 0.0857 | 0.0764 | 0.0716 | 0.0573 | 0.0668 |
| 0.040 | 0.0874 | 0.0778 | 0.0731 | 0.0585 | 0.0681 |
| 0.041 | 0.0890 | 0.0792 | 0.0747 | 0.0598 | 0.0695 |
| 0.042 | 0.0907 | 0.0807 | 0.0761 | 0.0609 | 0.0708 |
| 0.043 | 0.0923 | 0.0821 | 0.0775 | 0.0620 | 0.0721 |
| 0.044 | 0.0939 | 0.0836 | 0.0790 | 0.0632 | 0.0734 |
| 0.045 | 0.0954 | 0.0850 | 0.0803 | 0.0644 | 0.0747 |
| 0.046 | 0.0970 | 0.0863 | 0.0820 | 0.0656 | 0.0759 |
| 0.047 | 0.0985 | 0.0877 | 0.0835 | 0.0668 | 0.0772 |
| 0.048 | 0.1000 | 0.0890 | 0.0849 | 0.0679 | 0.0785 |
| 0.049 | 0.1015 | 0.0903 | 0.0864 | 0.0691 | 0.0797 |
| 0.050 | 0.1029 | 0.0916 | 0.0879 | 0.0703 | 0.0809 |

* *See* "Handbook," page 456.

TABLE* 24.

FORMULÆ, DESCRIPTIONS, MELTING POINTS AND SOLUBILITIES OF THE PRINCIPAL HYDRAZONES AND OSAZONES OF THE SUGARS.

*Phenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Methyldiose | Lactic aldehyde | $C_9H_{12}N_2O$ | Colorless plates | 93 | |
| Methyldiose | Acetol | $C_9H_{12}N_2O$ | Colorless crystals | 100–102 | Alcohol |
| Phenyltriose | ..... | $C_{15}H_{16}N_2O_2$ | Colorless needles | 170.5 | |
| Phenyltetrose | ..... | $C_{16}H_{18}N_2O_3$ | Shining leaflets | 154 | Ether, alcohol |
| Pentose | *l*-Arabinose | $C_{11}H_{16}N_2O_4$ | White crystals | 151–153 | Water |
| Pentose | *l*-Xylose | $C_{11}H_{16}N_2O_4$ | White needles | 116 | Very soluble in water |
| Pentose | *l*-Ribose | $C_{11}H_{16}N_2O_4$ | White crystals | 154–155 | Very soluble in water |
| Methylpentose | Fucose | $C_{12}H_{18}N_2O_4$ | White needles | 170–173 | Slightly in water |
| Methylpentose | Rhamnose | $C_{12}H_{18}N_2O_4$ | White leaflets | 156–159 | Water, alcohol |
| Hexose | *d*-Glucose | $\alpha C_{12}H_{18}N_2O_5$ | White leaflets | 159–160 | Water, alcohol |
| Hexose | *d*-Glucose | $\beta C_{12}H_{18}N_2O_5$ | White needles | 140–141 | Water, alcohol |
| Hexose | *d*-Mannose | $C_{12}H_{18}N_2O_5$ | White prisms | 195–200 | Hot water |
| Hexose | *l*-Mannose | $C_{12}H_{18}N_2O_5$ | White crystals | 195 | Hot water |
| Hexose | *d,l*-Mannose | $C_{12}H_{18}N_2O_5$ | White crystals | 195 | Hot water |
| Hexose | *d*-Galactose | $C_{12}H_{18}N_2O_5$ | White needles | 158 | Hot water |
| Hexose | *l*-Galactose | $C_{12}H_{18}N_2O_5$ | White crystals | 158–160 | Hot water |
| Hexose | *d,l*-Galactose | $C_{12}H_{18}N_2O_5$ | White leaflets | 158–160 | Hot water |
| Hexose | *d*-Fructose | $C_{12}H_{18}N_2O_5$ | White needles | ..... | Hot water |
| Hexose | *l*-Gulose | $C_{12}H_{18}N_2O_5$ | White needles | 143 | Hot water, alcohol |
| Hexose | *d,l*-Gulose | $C_{12}H_{18}N_2O_5$ | White needles | 143 | Slightly in water |
| Methylhexose | Rhamnohexose | $C_{13}H_{20}N_2O_5$ | ..... | ..... | Water |
| Methylhexose | $\alpha$-Rhodeohexose | $C_{13}H_{20}N_2O_5$ | Yellow plates | 150 | Alcohol |
| Methylhexose | $\beta$-Rhodeohexose | $C_{13}H_{20}N_2O_5$ | White plates | 131–137 | Alcohol |
| Heptose | $\alpha$-Glucoheptose | $C_{13}H_{20}N_2O_6$ | White needles | 170 | Water |
| Heptose | $\beta$-Glucoheptose | $C_{13}H_{20}N_2O_6$ | White needles | 190–193 | Water |
| Heptose | *d*-Mannoheptose | $C_{13}H_{20}N_2O_6$ | Fine white needles | 197–200 | Hot water |
| Heptose | *l*-Mannoheptose | $C_{13}H_{20}N_2O_6$ | Fine white needles | 196 | Hot water |
| Heptose | *d,l*-Mannoheptose | $C_{13}H_{20}N_2O_6$ | Fine white needles | 175–177 | |
| Heptose | $\alpha$-Galaheptose | $C_{13}H_{20}N_2O_6$ | Fine white needles | 200 | Hot water, alcohol |
| Methylheptose | Rhamnoheptose | $C_{14}H_{22}N_2O_6$ | Fine white needles | 200 | Hot water |
| Octose | $\alpha$-Glucooctose | $C_{14}H_{22}N_2O_7$ | Colorless prisms | 190 | Hot water |

* *See* "Handbook," page 353.

TABLE 24. (*Continued.*)

*Phenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Octose | *d*-Mannooctose | $C_{14}H_{22}N_2O_7$ | Fine white needles | 212 | Slightly in hot water |
| Octose | *d*-Galaoctose | $C_{14}H_{22}N_2O_7$ | Fine white leaflets | 200–205 | Hot water |
| Nonose | $\alpha$-Gluconononose | $C_{15}H_{24}N_2O_8$ | White crystals | 195–200 | Hot water |
| Nonose | *d*-Mannononose | $C_{15}H_{24}N_2O_8$ | White crystals | 223 | Hot water |
| Decose | $\alpha$-Glucodecose | $C_{16}H_{26}N_2O_9$ | Prismatic needles | 278 | |
| Disaccharide | Lactose | $C_{18}H_{28}N_2O_{10}$ | White hygroscopic crystals | ..... | Water, alcohol |
| Disaccharide | Maltose | $C_{18}H_{28}N_2O_{10}$ | White hygroscopic needles | 130 | Abs. alcohol |
| Disaccharide | Melibiose | $C_{18}H_{28}N_2O_{10}$ | Yellowish needles | 145 | Water |
| Disaccharide | Cellose | $C_{18}H_{28}N_2O_{10}$ | Yellow hygroscopic powder | ..... | Alcohol |
| Trisaccharide | Mannatrisaccharide | $C_{24}H_{38}N_2O_{15}$ | Yellow amorphous | ..... | Water, alcohol |

*Phenylosazones.*

| | | | | | |
|---|---|---|---|---|---|
| Diose | Glycolose | $C_{14}H_{14}N_4$ | Yellow leaflets | 179 | Hot alcohol |
| Methyldiose | Acetol | $C_{15}H_{16}N_4$ | Yellow leaflets | 145–148 | Benzol |
| Methyldiose | Lactic aldehyde | Identical with preceding | | | |
| Dimethyldiose | Dimethylglycolose | $C_{16}H_{18}N_4$ | Fine yellow crystals | 245 | Very slightly in ether |
| Triose | *d,l*-Glycerose | $C_{15}H_{16}N_4O$ | Yellow leaflets | 132 | Alcohol, ether |
| Triose | Dioxyacetone | Identical with preceding | | | |
| Methyltriose | Methylglycerose | $C_{16}H_{18}N_4O$ | Yellow crystals | 175.5 | Hot benzol |
| Tetrose | *d*-Erythrose | $C_{16}H_{18}N_4O_2$ | Yellow needles | 166–168 | Ether, benzol |
| Tetrose | *l*-Erythrose | $C_{16}H_{18}N_4O_2$ | Yellow needles | 164 | Benzol |
| Tetrose | *d,l*-Erythrose | $C_{16}H_{18}N_4O_2$ | Yellow needles | 164 | Alcohol, acetone |
| Tetrose | *l*-Threose | Identical with osazone of *d*-erythrose | | | |
| Tetrose | *d*-Erythrulose | Identical with osazone of *d*-erythrose | | | |
| Methyltetrose | Methyltetrose | $C_{17}H_{20}N_4O_2$ | Yellow needles | 173–174 | Alcohol |
| Oxymethyltetrose | Apiose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 156 | Hot water, alcohol |
| Pentose | *d*-Arabinose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 160–163 | Hot water |
| Pentose | *l*-Arabinose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 160 | Hot water, alcohol |
| Pentose | *d,l*-Arabinose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 166–168 | Hot water |
| Pentose | *l*-Xylose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 160–161 | Ether, acetone |
| Pentose | *d,l*-Xylose | $C_{17}H_{20}N_4O_3$ | Yellow needles | 210–215 | Slightly in hot alcohol |

TABLE 24. (*Continued.*)

*Phenylosazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Pentose | *d*-Lyxose | Identical with osazone of *l*-xylose | | | |
| Pentose | *l*-Ribose | Identical with osazone of *l*-arabinose | | | |
| Pentose | *d*-Araboketose | Identical with osazone of *d*-arabinose | | | |
| Pentose | *l*-Araboketose | Identical with osazone of *l*-arabinose | | | |
| Pentose | *d,l*-Xyloketose | Identical with osazone of *d,l*-xylose | | | |
| Methylpentose | Fucose | $C_{18}H_{22}N_4O_3$ | Yellow crystals | 177 | |
| Methylpentose | Rhodeose | $C_{18}H_{22}N_4O_3$ | Yellow crystals | 176.5 | Acetone |
| Methylpentose | Fucose+Rhodeose | $C_{18}H_{22}N_4O_3$ | Yellow crystals | 187 | |
| Methylpentose | Rhamnose | $C_{18}H_{22}N_4O_3$ | Yellow crystals | 180 | Acetone, pyridine |
| Methylpentose | Isorhamnose | Identical with preceding | | | |
| Methylpentose | Isorhodeose | $C_{18}H_{22}N_4O_3$ | Yellow prisms | 190 | Alcohol |
| Methylpentose | Quinovose | $C_{18}H_{22}N_4O_3$ | Yellow needles | 193–194 | Hot abs. alcohol |
| Hexose | *d*-Glucose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 204–205 | Hot alcohol |
| Hexose | *l*-Glucose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 205 | Hot alcohol |
| Hexose | *d,l*-Glucose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 217 | Glacial acetic acid |
| Hexose | *d*-Mannose | Identical with osazone of *d*-glucose | | | |
| Hexose | *l*-Mannose | Identical with osazone of *l*-glucose | | | |
| Hexose | *d,l*-Mannose | Identical with osazone of *d,l*-glucose | | | |
| Hexose | *d*-Fructose | Identical with osazones of *d*-glucose and *d*-mannose | | | |
| Hexose | *l*-Fructose | Identical with osazones of *l*-glucose and *l*-mannose | | | |
| Hexose | *d,l*-Fructose | Identical with osazones of *d,l*-glucose and *d,l*-mannose | | | |
| Hexose | *d*-Gulose | Identical with osazones of *d*-sorbose and *d*-idose | | | |
| Hexose | *l*-Gulose | $C_{18}H_{22}N_4O_4$ | Yellow flakes | 156 | Water, methyl alcohol |
| Hexose | *d,l*-Gulose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 157–159 | Slightly in hot water |
| Hexose | *d*-Idose | Identical with osazones of *d*-sorbose and *d*-gulose | | | |
| Hexose | *l*-Idose | Identical with osazones of *l*-sorbose and *l*-gulose | | | |
| Hexose | *d*-Galactose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 188–191<br>196–197 | 60% alcohol |
| Hexose | *l*-Galactose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 192–195 | |
| Hexose | *d,l*-Galactose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 206 | |
| Hexose | *d*-Talose | Identical with osazone of *d*-galactose | | | |

TABLE 24. (*Continued.*)

*Phenylosazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Hexose | *d*-Sorbose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 164 | Alcohol, methyl alcohol |
| Hexose | *l*-Sorbose | Identical with osazones of *l*-gulose and *l*-idose | | | |
| Hexose | Glutose | $C_{18}H_{22}N_4O_4$ | Needles | 165 | Methyl alcohol |
| Hexose | *d*-Tagatose | Identical with osazone of *d*-galactose | | | |
| Hexose | *l*-Tagatose | Identical with osazone of *l*-galactose | | | |
| Hexose | Galtose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 182 | Methyl alcohol |
| Hexose | Formose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 144 | Hot alcohol |
| Hexose | $\alpha$-Acrose | Identical with osazone of *d*,*l*-glucose | | | |
| Hexose | $\beta$-Acrose | $C_{18}H_{22}N_4O_4$ | Yellow needles | 158–159 | Alcohol, acetone |
| Methylhexose | $\alpha$-Rhamnohexose | $C_{19}H_{24}N_4O_4$ | Yellow needles | 200 | Hot alcohol |
| Methylhexose | $\beta$-Rhamnohexose | Identical with preceding | | | |
| Methylhexose | $\alpha$-Rhodeohexose | $C_{19}H_{24}N_4O_4$ | Yellow needles | 231 | Acetone |
| Methylhexose | $\beta$-Rhodeohexose | $C_{19}H_{24}N_4O_4$ | Yellow powder | 213 | Alcohol |
| Heptose | $\alpha$-Glucoheptose | $C_{19}H_{24}N_4O_5$ | Yellow needles | 195 | Pyridine-alcohol |
| Heptose | $\beta$-Glucoheptose | Identical with preceding | | | |
| Heptose | *d*-Mannoheptose | $C_{19}H_{24}N_4O_5$ | Yellow needles | 200 | Hot alcohol |
| Heptose | *l*-Mannoheptose | $C_{19}H_{24}N_4O_5$ | Yellow needles | 203 | Hot alcohol |
| Heptose | *d*,*l*-Mannoheptose | $C_{19}H_{24}N_4O_5$ | Yellow needles | 210 | |
| Heptose | $\alpha$-Galaheptose | $C_{19}H_{24}N_4O_5$ | Yellow needles | 218 | Hot alcohol |
| Heptose | Volemose | $C_{19}H_{24}N_4O_5$ | Yellow crystals | 196 | Hot alcohol |
| Methylheptose | Rhamnoheptose | $C_{20}H_{26}N_4O_5$ | Yellow needles | 200 | Slightly in hot alcohol |
| Octose | $\alpha$-Glucooctose | $C_{20}H_{26}N_4O_6$ | Yellow crystals | 210–212 | Hot alcohol |
| Octose | *d*-Mannooctose | $C_{20}H_{26}N_4O_6$ | Yellow needles | 223 | |
| Octose | *d*-Galaoctose | $C_{20}H_{26}N_4O_6$ | Yellow needles | 220–225 | |
| Methyloctose | Rhamnooctose | $C_{21}H_{28}N_4O_6$ | ..... | 216 | |
| Nonose | $\alpha$-Glucononose | $C_{21}H_{28}N_4O_7$ | Yellow needles | 220–223 | Very slightly in water |
| Nonose | *d*-Mannononose | $C_{21}H_{28}N_4O_7$ | Yellow needles | 217 | Very slightly in water |
| Disaccharide | Galactoarabinose | $C_{23}H_{30}N_4O_8$ | Needles | 236–238 | Hot water |
| Disaccharide | Maltose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 202–208 | Hot water |
| Disaccharide | Isomaltose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 140–158 | Hot water |
| Disaccharide | Lactose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 200 | Hot water |

TABLE 24. (*Continued.*)

*Phenylosazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Disaccharide | Isolactose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 190–193 | |
| Disaccharide | Turanose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 215–220 | Hot water |
| Disaccharide | Melibiose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 176–178 | Hot water |
| Disaccharide | Gentiobiose | $C_{24}H_{32}N_4O_9$ | ..... | 142 | |
| Disaccharide | Cellose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 198 | Alcohol, hot water |
| Disaccharide | Glucosidogalactose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 172–174 | Hot water |
| Disaccharide | Galactosidogalactose | $C_{24}H_{32}N_4O_9$ | Yellow needles | 173–175 | Hot water |
| Trisaccharide | Mannatrisaccharide | $C_{30}H_{42}N_4O_{14}$ | Microscopic needles | 122 | Hot water |

*p-Bromophenylhydrazones.*

| | | | | | |
|---|---|---|---|---|---|
| Pentose | *d*-Arabinose | $C_{11}H_{15}BrN_2O_4$ | White needles | 163 | Hot alcohol |
| Pentose | *l*-Arabinose | $C_{11}H_{15}BrN_2O_4$ | White needles | 162 | Hot alcohol |
| Pentose | *d,l*-Arabinose | $C_{11}H_{15}BrN_2O_4$ | White needles | 160 | Pyridine |
| Pentose | *l*-Xylose | $C_{11}H_{15}BrN_2O_4$ | Yellowish crystals | 128 | Hot water |
| Pentose | *l*-Ribose | $C_{11}H_{15}BrN_2O_4$ | White crystals | 164–165 | Hot water |
| Methylpentose | Rhamnose | $C_{12}H_{17}BrN_2O_4$ | Rhombic crystals | 160, 167 | Hot water |
| Methylpentose | Fucose | $C_{12}H_{17}BrN_2O_4$ | Glossy scales | 181–183 | 50% alcohol |
| Methylpentose | Rhodeose | $C_{12}H_{17}BrN_2O_4$ | Glossy scales | 184 | Hot alcohol |
| Hexose | *d*-Glucose | $C_{12}H_{17}BrN_2O_5$ | ..... | 147, 166 | Pyridine |
| Hexose | *d*-Mannose | $C_{12}H_{17}BrN_2O_5$ | Glossy leaflets | 208–210 | Glacial acetic acid |
| Hexose | *d*-Galactose | $C_{12}H_{17}BrN_2O_5$ | ..... | 168 | |
| Methylhexose | $\alpha$-Rhodeohexose | $C_{13}H_{19}BrN_2O_5$ | White powder | 173 | Acetone |
| Methylhexose | $\beta$-Rhodeohexose | $C_{13}H_{19}BrN_2O_5$ | White scales | 145 | Alcohol |
| Heptose | $\alpha$-Glucoheptose | $C_{13}H_{19}BrN_2O_6$ | | 158 | |

*p-Bromophenylosazones.*

| | | | | | |
|---|---|---|---|---|---|
| Triose | *d,l*-Glycerose | $C_{15}H_{14}Br_2N_4O$ | Yellow needles | 168 | Ether, acetone |
| Tetrose | *d*-Erythrose | $C_{16}H_{16}Br_2N_4O_2$ | Yellow needles | 195 | |
| Oxymethyltetrose | Apiose | $C_{17}H_{18}Br_2N_4O_3$ | Yellow needles | 209–212 | Alcohol |

TABLE 24. (*Continued.*)

*p-Bromophenylosazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Pentose | *l*-Arabinose | $C_{17}H_{18}Br_2N_4O_3$ | Yellow needles | 196–200 | Hot water, alcohol |
| Pentose | *d,l*-Arabinose | $C_{17}H_{18}Br_2N_4O_3$ | Yellow needles | 200–202 | |
| Pentose | *l*-Xylose | $C_{17}H_{18}Br_2N_4O_3$ | Yellow needles | 208 | Pyridine |
| Methylpentose | Rhamnose | $C_{18}H_{20}Br_2N_4O_3$ | Yellow needles | 215 | Alcohol |
| Methylpentose | Isorhodeose | $C_{18}H_{20}Br_2N_4O_3$ | Yellow needles | 183–184 | |
| Hexose | *d*-Glucose | $C_{18}H_{20}Br_2N_4O_4$ | Yellow needles | 222 | Pyridine |
| Hexose | *d*-Gulose | $C_{18}H_{20}Br_2N_4O_4$ | Identical with *d*-sorbose-*p*-bromophenylosazone | | |
| Hexose | *d,l*-Gulose | $C_{18}H_{20}Br_2N_4O_4$ | Yellow needles | 180–183 | Acetic ether |
| Hexose | *d*-Sorbose | $C_{18}H_{20}Br_2N_4O_4$ | Yellow needles | 181 | Hot water |
| Hexose | $\beta$-Acrose | $C_{18}H_{20}Br_2N_4O_4$ | Yellow needles | 180–183 | Acetic ether |
| Methylhexose | $\alpha$-Rhodeohexose | $C_{19}H_{22}Br_2N_4O_4$ | Yellow scales | 219 | Alcohol |
| Methylhexose | $\beta$-Rhodeohexose | $C_{19}H_{22}Br_2N_4O_4$ | Yellow powder | 200 | Alcohol |
| Disaccharide | Maltose | $C_{24}H_{30}Br_2N_4O_9$ | Yellow needles | 198 | Alcohol, acetone |
| Disaccharide | Melibiose | $C_{24}H_{30}Br_2N_4O_9$ | Yellow needles | 181–182 | Hot alcohol |

*p-Nitrophenylhydrazones.*

| | | | | | |
|---|---|---|---|---|---|
| Pentose | *l*-Arabinose | $C_{11}H_{15}N_3O_6$ | Yellow crystalline powder | 181–182 | Slightly in alcohol |
| Pentose | *l*-Xylose | $C_{11}H_{15}N_3O_6$ | Yellow crystals | 156 | Alcohol |
| Methylpentose | Rhamnose | $C_{12}H_{17}N_3O_6$ | Yellow needles | 186 | |
| Hexose | *d*-Glucose | $\alpha C_{12}H_{17}N_3O_7$ | Yellow needles | 185–188 | Pyridine+methyl alcohol |
| Hexose | *d*-Glucose | $\beta C_{12}H_{17}N_3O_7$ | Yellow needles | 195 | |
| Hexose | *d*-Mannose | $\alpha C_{12}H_{17}N_3O_7$ | Yellow needles | 190 | |
| Hexose | *d*-Mannose | $\beta C_{12}H_{17}N_3O_7$ | Yellow needles | 202 | |
| Hexose | *d*-Fructose | $C_{12}H_{17}N_3O_7$ | Yellow needles | 176 | Pyridine+methyl alcohol |
| Hexose | *d*-Galactose | $C_{12}H_{17}N_3O_7$ | Yellow needles | 192 | Pyridine+methyl alcohol |

*p-Nitrophenylosazones.*

| | | | | | |
|---|---|---|---|---|---|
| Diose | Glycolose | $C_{14}H_{12}N_6O_4$ | Red needles | 311 | Pyridine |
| Methylpentose | Rhamnose | $C_{18}H_{20}N_6O_7$ | Red needles | 208 | In NaOH with blue color |

TABLE 24. (*Continued.*)

*p-Nitrophenylosazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Hexose | *d*-Glucose | $C_{18}H_{20}N_6O_8$ | Red needles | 257 | Pyridine+methyl alcohol |
| Hexose | *d*-Fructose | Identical with preceding | | | |
| Disaccharide | Maltose | $C_{24}H_{30}N_6O_{13}$ | Red needles | 261 | |
| Disaccharide | Lactose | $C_{24}H_{30}N_6O_{13}$ | Red needles | 258 | In NaOH with blue color |
| | | *o-Nitrophenylhydrazones.* | | | |
| Pentose | *l*-Arabinose | $C_{11}H_{15}N_3O_6$ | Red-yellow crystals | 180 | Slightly in alcohol |
| Methylpentose | Rhamnose | $C_{12}H_{17}N_3O_6$ | Yellow crystalline powder | 151 | |
| Hexose | *d*-Glucose | $C_{12}H_{17}N_3O_7$ | Yellow crystals | 148 | Alcohol |
| Hexose | *d*-Mannose | $C_{12}H_{17}N_3O_7$ | Yellow crystals | 173 | Slightly in methyl alcohol |
| Hexose | *d*-Galactose | $C_{12}H_{17}N_3O_7$ | Red-yellow crystals | 172 | Slightly in alcohol |
| Hexose | *d*-Fructose | $C_{12}H_{17}N_3O_7$ | Brick-red powder | 155–156 | Methyl alcohol |
| | | *o-Nitrophenylosazones.* | | | |
| Hexose | *d*-Glucose | $C_{18}H_{20}N_6O_8$ | Brick red powder | 215–217 | Very slightly in alcohol |
| | | *m-Nitrophenylhydrazones.* | | | |
| Pentose | *l*-Arabinose | $C_{11}H_{15}N_3O_6$ | Red-yellow crystals | 179–180 | Slightly in alcohol |
| Methylpentose | Rhamnose | $C_{12}H_{17}N_3O_6$ | Red-yellow crystals | 104–105 | Alcohol |
| Hexose | *d*-Glucose | $C_{12}H_{17}N_3O_7$ | Yellow crystals | 115–116 | Alcohol |
| Hexose | *d*-Mannose | $C_{12}H_{17}N_3O_7$ | Yellow crystals | 162–163 | Alcohol |
| Hexose | *d*-Galactose | $C_{12}H_{17}N_3O_7$ | Yellow crystals | 181–182 | Slightly in alcohol |
| | | *m-Nitrophenylosazones.* | | | |
| Hexose | *d*-Glucose | $C_{18}H_{20}N_6O_8$ | Red-brown powder | 228 | Very slightly in alcohol |

TABLE 24. (*Continued.*)

*Methylphenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Triose | *d,l*-Glycerose | $C_{10}H_{14}N_2O_2$ | White needles | 120 | Hot water, alcohol |
| Pentose | *l*-Arabinose | $C_{12}H_{18}N_2O_4$ | White crystals | 161–164 | Alcohol, pyridine |
| Pentose | *d,l*-Arabinose | $C_{12}H_{18}N_2O_4$ | White leaflets | 173 | Water, alcohol, pyridine |
| Pentose | *l*-Xylose | $C_{12}H_{18}N_2O_4$ | White leaflets | 103–110 | Water, alcohol, pyridine |
| Methylpentose | Rhamnose | $C_{13}H_{20}N_2O_4$ | White crystals | 124 | Abs. methyl alcohol |
| Methylpentose | Fucose | $C_{13}H_{20}N_2O_4$ | White needles | 177 | Alcohol, pyridine |
| Methylpentose | Rhodeose | $C_{13}H_{20}N_2O_4$ | White needles | 181 | Hot water, alcohol |
| Hexose | *d*-Glucose | $C_{13}H_{20}N_2O_5$ | Long white leaflets | 130 | Alcohol |
| Hexose | *d*-Mannose | $C_{13}H_{20}N_2O_5$ | White crystals | 178 | Methyl alcohol |
| Hexose | *d*-Galactose | $C_{13}H_{20}N_2O_5$ | White crystals | 188–191 | Methyl alcohol |
| Hexose | *d,l*-Galactose | $C_{13}H_{20}N_2O_5$ | White crystals | 183 | Hot water |
| Hexose | *d*-Talose | $C_{13}H_{20}N_2O_5$ | White crystals | 154 | Methyl alcohol |
| Hexose | *d*-Fructose | $C_{13}H_{20}N_2O_5$ | Prisms | 116–120 | Alcohol |
| Methylhexose | $\alpha$-Rhodeohexose | $C_{14}H_{22}N_2O_5$ | White scales | 188 | Acetone |
| Methylhexose | $\beta$-Rhodeohexose | $C_{14}H_{22}N_2O_5$ | Silvery lustrous plates | 163 | Alcohol |
| Heptose | $\alpha$-Glucoheptose | $C_{14}H_{22}N_2O_6$ | Fine needles | 150 | Alcohol |
| | | *Methylphenylosazones.* | | | |
| Triose | Dioxyacetone | $C_{17}H_{20}N_4O$ | Yellow needles | 127–130 | Alcohol, pyridine |
| Tetrose | *d,l*-Erythrulose | $C_{18}H_{22}N_4O_2$ | Yellow needles | 158–159 | Alcohol, pyridine |
| Pentose | *d*-Araboketose | $C_{19}H_{24}N_4O_3$ | Yellow needles | 173 | Pyridine |
| Pentose | *d,l*-Xyloketose | $C_{19}H_{24}N_4O_3$ | Yellow needles | 173 | Pyridine |
| Pentose | *d,l*-Riboketose | $C_{19}H_{24}N_4O_3$ | Yellow needles | 175 | Pyridine |
| Hexose | *d*-Fructose | $C_{20}H_{26}N_4O_4$ | Yellow needles | 158–160 | Pyridine |
| Hexose | *d,l*-Fructose | $C_{20}H_{26}N_4O_4$ | Yellow needles | 158 | 10% alcohol |
| Hexose | *d*-Sorbose | $C_{20}H_{26}N_4O_4$ | Yellow oil | ..... | Alcohol |
| Hexose | *d,l*-Tagatose | $C_{20}H_{26}N_4O_4$ | Yellow needles | 148–150 | Pyridine-water |

TABLE 24. (*Continued.*)

*Ethylphenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Pentose | *l*-Arabinose | $C_{13}H_{20}N_2O_4$ | Yellow needles | 153 | Methyl alcohol |
| Methylpentose | Rhamnose | $C_{14}H_{22}N_2O_4$ | Yellow needles | 123 | Methyl alcohol |
| Methylpentose | Rhodeose | $C_{14}H_{22}N_2O_4$ | White needles | 193 | 96% alcohol |
| Hexose | *d*-Mannose | $C_{14}H_{22}N_2O_5$ | Yellow needles | 159 | Methyl alcohol |
| Hexose | *d*-Galactose | $C_{14}H_{22}N_2O_5$ | White needles | 169 | Methyl alcohol |
| | | *Allylphenylhydrazones.* | | | |
| Pentose | *l*-Arabinose | $C_{14}H_{20}N_2O_4$ | Yellow needles | 145 | Methyl alcohol |
| Methylpentose | Rhamnose | $C_{15}H_{22}N_2O_4$ | Yellow needles | 135 | Methyl alcohol |
| Hexose | *d*-Glucose | $C_{15}H_{22}N_2O_5$ | Yellow needles | 155 | Methyl alcohol |
| Hexose | *d*-Mannose | $C_{15}H_{22}N_2O_5$ | Yellow needles | 142 | Methyl alcohol |
| Hexose | *d*-Galactose | $C_{15}H_{22}N_2O_5$ | Yellow needles | 157 | Methyl alcohol |
| Disaccharide | Lactose | $C_{21}H_{32}N_2O_{10}$ | Yellow needles | 132 | Methyl alcohol |
| Disaccharide | Melibiose | $C_{21}H_{32}N_2O_{10}$ | Yellow needles | 197 | Methyl alcohol |
| | | *Amylphenylhydrazones.* | | | |
| Pentose | *l*-Arabinose | $C_{16}H_{26}N_2O_4$ | Yellow needles | 120 | Methyl alcohol |
| Methylpentose | Rhamnose | $C_{17}H_{28}N_2O_4$ | Brown crystals | 99 | Methyl alcohol |
| Hexose | *d*-Glucose | $C_{17}H_{28}N_2O_5$ | Brown needles | 128 | Methyl alcohol |
| Hexose | *d*-Mannose | $C_{17}H_{28}N_2O_5$ | Yellow needles | 134 | Methyl alcohol |
| Hexose | *d*-Galactose | $C_{17}H_{28}N_2O_5$ | Yellow needles | 116 | Methyl alcohol |
| Disaccharide | Lactose | $C_{23}H_{38}N_2O_{10}$ | Brown needles | 123 | Methyl alcohol |
| | | *d-Amylphenylhydrazones.* | | | |
| Pentose | *d*-Arabinose | $C_{16}H_{26}N_2O_4$ | White needles | 115 | Water, alcohol |
| Pentose | *l*-Arabinose | $C_{16}H_{26}N_2O_4$ | White needles | 127 | Alcohol |

TABLE 24. (*Continued.*)

*Diphenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Triose | *d,l*-Glycerose | $C_{15}H_{16}N_2O_2$ | Needles | 133 | |
| Pentose | *d*-Arabinose | $C_{17}H_{20}N_2O_4$ | White needles | 198 | Pyridine |
| Pentose | *l*-Arabinose | $C_{17}H_{20}N_2O_4$ | White needles | 204–205 | Pyridine |
| Pentose | *d,l*-Arabinose | $C_{17}H_{20}N_2O_4$ | White needles | 206 | Pyridine |
| Pentose | *l*-Xylose | $C_{17}H_{20}N_2O_4$ | White needles | 128 | |
| Methylpentose | Rhamnose | $C_{18}H_{22}N_2O_4$ | White prisms | 134 | Water, alcohol |
| Methylpentose | Fucose | $C_{18}H_{22}N_2O_4$ | White needles | 198–199 | Hot alcohol |
| Methylpentose | Rhodeose | $C_{18}H_{22}N_2O_4$ | White needles | 199 | Hot alcohol |
| Hexose | *d*-Glucose | $C_{18}H_{22}N_2O_5$ | White crystals | 161 | Hot water |
| Hexose | *l*-Glucose | $C_{18}H_{22}N_2O_5$ | White crystals | 162 | Hot water |
| Hexose | *d,l*-Glucose | $C_{18}H_{22}N_2O_5$ | White crystals | 132–133 | |
| Hexose | *d*-Mannose | $C_{18}H_{22}N_2O_5$ | White prisms | 155 | |
| Hexose | *d*-Galactose | $C_{18}H_{22}N_2O_5$ | White crystals | 157 | Hot water |
| Heptose | α-Glucoheptose | $C_{19}H_{24}N_2O_6$ | White crystals | 140 | |

*Diphenylosazones.*

| | | | | | |
|---|---|---|---|---|---|
| Diose | Glycolose | $C_{26}H_{22}N_4$ | ..... | 207 | |
| Hexose | *d*-Fructose | $C_{30}H_{30}N_4O_4$ | Yellow needles | 167 | |

*Benzylphenylhydrazones.*

| | | | | | |
|---|---|---|---|---|---|
| Methyltriose | Methylglycerose | $C_{17}H_{20}N_2O_2$ | Colorless needles | 116 | Cold alcohol |
| Tetrose | *d*-Erythrose | $C_{17}H_{20}N_2O_3$ | White needles | 105.5 | 95% alcohol |
| Tetrose | *l*-Erythrose | $C_{17}H_{20}N_2O_3$ | White needles | 105 | 95% alcohol |
| Tetrose | *d,l*-Erythrose | $C_{17}H_{20}N_2O_3$ | White needles | 83 | |
| Tetrose | *l*-Threose | $C_{17}H_{20}N_2O_3$ | Needles | 194.5 | Benzol |
| Methyltetrose | Methyltetrose | $C_{18}H_{22}N_2O_3$ | White needles | 96–97 | Alcohol |
| Pentose | *d*-Arabinose | $C_{18}H_{22}N_2O_4$ | White needles | 174 | Methyl alcohol |
| Pentose | *l*-Arabinose | $C_{18}H_{22}N_2O_4$ | White needles | 170 | Methyl alcohol |

TABLE 24. (*Concluded.*)

*Benzylphenylhydrazones.*

| Class. | Sugar. | Formula. | Description. | Melting point ° C. | Solubility. |
|---|---|---|---|---|---|
| Pentose | *d,l*-Arabinose | $C_{18}H_{22}N_2O_4$ | Yellow needles | 185 | Pyridine |
| Pentose | *l*-Xylose | $C_{18}H_{22}N_2O_4$ | White needles | 99 | Alcohol |
| Pentose | *d*-Lyxose | $C_{18}H_{22}N_2O_4$ | Fine needles | 116 | Alcohol |
| Methylpentose | Rhamnose | $C_{19}H_{24}N_2O_4$ | Yellow crystals | 121 | Absolute alcohol |
| Methylpentose | Fucose | $C_{19}H_{24}N_2O_4$ | Yellow crystals | 173 | Pyridine |
| Methylpentose | Rhodeose | $C_{19}H_{24}N_2O_4$ | White needles | 179 | Hot alcohol |
| Hexose | *d*-Glucose. | $C_{19}H_{24}N_2O_5$ | Yellow needles | 163–165 | Pyridine |
| Hexose | *d*-Mannose | $C_{19}H_{24}N_2O_5$ | White needles | 165 | Methyl alcohol |
| Hexose | *l*-Gulose | $C_{19}H_{24}N_2O_5$ | Yellow needles | 124 | Methyl alcohol |
| Hexose | *d*-Galactose | $C_{19}H_{24}N_2O_5$ | Yellow needles | 154–158 | Methyl alcohol |
| Disaccharide | Galactoarabinose | $C_{24}H_{32}N_2O_9$ | White leaflets | ..... | Water, alcohol |
| Disaccharide | Lactose | $C_{25}H_{34}N_2O_{10}$ | Yellow needles | 128 | Methyl alcohol |
| | | *Benzylphenylosazones.* | | | |
| Diose | Glycolose | $C_{28}H_{26}N_4$ | Yellow needles | 197.5 | |
| Hexose | *d*-Fructose | $C_{32}H_{34}N_4O_4$ | Yellow needles | 190.0 | Pyridine-alcohol |
| | | *β-Naphthylhydrazones.* | | | |
| Pentose | *l*-Arabinose (a) | $C_{15}H_{18}N_2O_4$ | Brown needles | 141 | Methyl alcohol |
| Pentose | *l*-Arabinose (b) | $C_{15}H_{18}N_2O_4$ | White needles | 176–177 | |
| Pentose | *l*-Xylose (a) | $C_{15}H_{18}N_2O_4$ | Brown needles | 70 | Methyl alcohol |
| Pentose | *l*-Xylose (b) | $C_{15}H_{18}N_2O_4$ | White needles | 123–124 | |
| Methylpentose | Rhamnose | $C_{16}H_{20}N_2O_4$ | Brown needles | 170 | Methyl alcohol |
| Hexose | *d*-Glucose | $C_{16}H_{20}N_2O_5$ | Yellow needles | 178–179 | Hot alcohol |
| Hexose | *d*-Mannose (a) | $C_{16}H_{20}N_2O_5$ | Brown needles | 157 | Methyl alcohol |
| Hexose | *d*-Mannose (b) | $C_{16}H_{20}N_2O_5$ | White crystals | 186 | |
| Hexose | *d*-Galactose (a) | $C_{16}H_{20}N_2O_5$ | Brown needles | 167 | Methyl alcohol |
| Hexose | *d*-Galactose (b) | $C_{16}H_{20}N_2O_5$ | White crystals | 190 | Methyl alcohol |
| Hexose | *d*-Fructose | $C_{16}H_{20}N_2O_5$ | Yellow needles | 162 | Methyl alcohol |
| Disaccharide | Maltose | $C_{22}H_{30}N_2O_{10}$ | Brown crystals | 176 | Methyl alcohol |
| Disaccharide | Lactose | $C_{22}H_{30}N_2O_{10}$ | Brown needles | 203 | Glacial acetic acid |
| Disaccharide | Melibiose | $C_{22}H_{30}N_2O_{10}$ | Brown needles | 135 | Methyl alcohol |

TABLE 25.

RECIPROCALS OF NUMBERS FROM 1 TO 100.

| Number. | Reciprocal. | Number. | Reciprocal. | Number. | Reciprocal. | Number. | Reciprocal. |
|---|---|---|---|---|---|---|---|
| 1 | 1.0000 | 26 | 0.0385 | 51 | 0.0196 | 76 | 0.0132 |
| 2 | 0.5000 | 27 | 0.0370 | 52 | 0.0192 | 77 | 0.0130 |
| 3 | 0.3333 | 28 | 0.0357 | 53 | 0.0189 | 78 | 0.0128 |
| 4 | 0.2500 | 29 | 0.0345 | 54 | 0.0185 | 79 | 0.0127 |
| 5 | 0.2000 | 30 | 0.0333 | 55 | 0.0182 | 80 | 0.0125 |
| 6 | 0.1667 | 31 | 0.0323 | 56 | 0.0179 | 81 | 0 0123 |
| 7 | 0.1429 | 32 | 0.0313 | 57 | 0.0175 | 82 | 0.0122 |
| 8 | 0.1250 | 33 | 0.0303 | 58 | 0.0172 | 83 | 0.0120 |
| 9 | 0.1111 | 34 | 0.0294 | 59 | 0.0169 | 84 | 0.0119 |
| 10 | 0.1000 | 35 | 0.0286 | 60 | 0.0167 | 85 | 0.0118 |
| 11 | 0.0909 | 36 | 0.0278 | 61 | 0.0164 | 86 | 0.0116 |
| 12 | 0.0833 | 37 | 0.0270 | 62 | 0.0161 | 87 | 0.0115 |
| 13 | 0.0769 | 38 | 0.0263 | 63 | 0.0159 | 88 | 0.0114 |
| 14 | 0.0714 | 39 | 0.0256 | 64 | 0.0156 | 89 | 0.0112 |
| 15 | 0.0667 | 40 | 0.0250 | 65 | 0.0154 | 90 | 0.0111 |
| 16 | 0.0625 | 41 | 0.0244 | 66 | 0.0152 | 91 | 0.0110 |
| 17 | 0.0588 | 42 | 0.0238 | 67 | 0.0149 | 92 | 0.0109 |
| 18 | 0.0555 | 43 | 0.0233 | 68 | 0.0147 | 93 | 0.0108 |
| 19 | 0.0526 | 44 | 0.0227 | 69 | 0.0145 | 94 | 0.0106 |
| 20 | 0.0500 | 45 | 0.0222 | 70 | 0.0143 | 95 | 0.0105 |
| 21 | 0.0476 | 46 | 0.0217 | 71 | 0.0141 | 96 | 0.0104 |
| 22 | 0.0455 | 47 | 0.0213 | 72 | 0.0139 | 97 | 0.0103 |
| 23 | 0.0435 | 48 | 0.0208 | 73 | 0.0137 | 98 | 0.0102 |
| 24 | 0.0417 | 49 | 0.0204 | 74 | 0.0135 | 99 | 0.0101 |
| 25 | 0.0400 | 50 | 0.0200 | 75 | 0.0133 | 100 | 0.0100 |

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